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Studies of Methods and Materials for the Control of the Leafhopper Empoasca fabae as a Bean Pest

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Studies of Methods and Materials for the Control of the Leafhopper Empoasca fabae as a Bean Pest[1]

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INTRODUCTION

The present studies on the control of the potato leafhopper (*Empoasca fabae* (Harr.)) as a pest of beans were made mainly at Columbus, Ohio, in 1926, 1927, and 1928, with additional studies in Florida and Ohio in 1932 and 1933.

The following three different phases of experimental procedure have been carried out in these studies: (1) Field tests on plots have been performed to determine the relative value of various materials under conditions of normal infestations on beans and where the practical control can be observed most successfully, (2) tests have been made upon single bean plants with several materials to gain a knowledge of their ovicidal and insecticidal properties, and (3) special and detailed studies have been made of bordeaux mixture and sulfur products and of the temporary insecticidal effect produced by them through the plant. The plot tests were made upon the plantings on the farm of the Ohio State University or one of the other State institutions in Columbus. The individual-plant tests were made in the insectary described in an earlier bulletin (*16*).[3]

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[1] Submitted for publication July 18, 1939.
[2] Field assistant during the summers of 1924–32.
[3] Italic numbers in parentheses refer to Literature Cited, p. 60.

MATERIALS USED IN PRELIMINARY STUDIES

The series of experiments performed in 1926, 1927, and 1928 [4] showed best control with bordeaux mixture and pyrethrum products. From the standpoint of immediate killing the pyrethrum is the better of the two, for all the nymphs and adults reached by the insecticide are usually killed in a few minutes' time, but it has no lasting toxic qualities. From the standpoint of seasonal control the bordeaux mixture is better, for it has an effect for several days on the nymphs that hatch after its application, although it is much slower in its toxic effects.

The nicotine-soap and nicotine-oil combinations were never satisfactory, even with different methods of application and when applied under different climatic conditions. A partial control was usually effected, but in no case a commercial control. Where the leafhoppers were temporarily stupefied they revived.

Derris extract proved less effective than either pyrethrum or nicotine.

Very promising results were obtained with monohydrated copper sulfate dust, which gave a marked reduction in the populations but was in no case as good as bordeaux mixture. In the dry dust form it seems to repel the leafhoppers, which, soon after its application, leave the plants; but when the dust becomes wet upon the plants it seems to act very much like bordeaux spray, and the leafhoppers remain on the plants and die as a result of feeding there.

When these studies were first begun many materials were used in the field to determine their relative toxicity. Following this, many materials were used in the insectary. Table 1 summarizes the field tests of these 3 years.

TABLE 1.—*Field insecticide tests against Empoasca fabae on bean plants, 1926–28* [1]

Treatment	Results obtained	Remarks
Nicotine sulfate [2] solution 1 pint and miscible oil [3] 1 gallon to 100 gallons of water.	About 60-percent control	2 or 3 nymphs per leaf alive.
Nicotine sulfate [2] solution 1 pint and 2 pounds of white laundry soap to 100 gallons of water.	Not satisfactory control	Large nymphs and adults not killed.
Nicotine sulfate [2] solution 1 pint and commercial white oil emulsion [4] 2 gallons to 100 gallons of water.	High mortality	Average 2 or 3 live nymphs per plant.
Commercial white oil emulsion [4] 2 percent.	Not satisfactory control	Many large nymphs and adults not killed.
Bordeaux mixture:		
2–4–50	High mortality	Very few nymphs alive.
3–3–50	do	Do.
3–6–50	do	Do.
4–4–50	do	Do.
4–6–50	do	Do.
5–8–50	do	Do.
2–4–50 plus arsenate of lead 1–50	do	Do.
3–3–50 plus arsenate of lead 1–50	do	Less than 1 live nymph per plant.
3–6–50 plus arsenate of lead 1–50	do	Plant injury 10–20 percent.
4–4–50 plus arsenate of lead 1½–50	do	Very few nymphs alive.
4–6–50 plus arsenate of lead 1–50	do	Less than 1 live nymph per plant.
5–8–50 plus arsenate of lead 1–50	do	Do.

[1] Tests were performed between June 23 and Aug. 19 during 1926, 1927, and 1928, and results were based on examinations made 4 days to 2 weeks after treatment.
[2] Containing 40 percent of free nicotine.
[3] A proprietary material.
[4] Refined white oil, unsulfonatable residue 98 percent, viscosity 75–80 percent.

[4] Since the results of these experiments were put in manuscript form, in October 1931, data obtained in 1932 and 1933 have been added.

TABLE 1.—*Field insecticide tests against Empoasca fabae on bean plants, 1926–28*—Continued

Treatment	Results obtained	Remarks
Pyrethrum spray:		
Japanese beetle formula [5] 1–15	do	No nymphs or adults remained alive.
1–19	do	Do.
1–38	do	Do.
1–76	do	Do.
1–152	do	Do.
1–200	do	Do.
1–250	do	Average 1 large nymph to a plant.
1–304	do	Very few nymphs alive.
1–400	do	Do.
Modified pyrethrum spray, 1 gallon of modified Japanese beetle formula [6] to 200 gallons of water.	do	Do.
Modified pyrethrum spray [6] 1–300	do	Do.
Commercial pyrethrum spray [5] ½ ounce to 1 gallon of water.	do	Do.
Commercial pyrethrum spray [5] ¼ ounce to 1 gallon of water.	do	Do.
Commercial pyrethrum extract [7] 1 ounce to 6 gallons of water.	do	Do.
Commercial pyrethrum extract [7] 1 ounce to 12 gallons of water.	do	Do.
Commercial pyrethrum extract [7] 1 ounce to 24 gallons of water.	Not satisfactory	Many large nymphs and adults not killed.
Monohydrated copper sulfate, hydrated lime 20–80 (applied to dry plants).	High mortality	Very few nymphs alive.
Do	Partial control only	Several nymphs per plant alive.
Do	High mortality	A few nymphs per plant but very marked reduction. Not as good as when used on wet foliage.
Do	Not satisfactory, partial control.	
Monohydrated copper sulfate, hydrated lime 20–80 (applied to wet plants)[8].	High mortality, at least 90 percent control.	Seems to act like bordeaux spray when applied to wet plants.
Sulfur dust [9]	Partial control	High percentage of large nymphs killed.
Sodium fluosilicate [10] spray 2 pounds to 50 gallons of water.	Not satisfactory	Population not reduced.
Sodium fluosilicate [10] (dust)	do	Do.
Sodium fluoride, bentonite 1–2 (dust)	do	Do.
Sodium fluoride, diatomaceous earth 1–2 (dust).	do	Do.
Sodium fluoride, hydrated lime 1–2 (dust).	do	Do.
Arsenate of lead [11], monohydrated copper sulfate, hydrated lime 1–1–8 [12] (dust).	Partial control, about 60 percent killed	
Thiocarbanilide preparation and flour 2–4–50 [13] (dust).	Not satisfactory	No reduction in population.
"Calcium fluosilicate compound" (dust)	do	Do.
Calcium cyanide (dust)	High mortality	10 percent plant injury.
Check		Average of 60 nymphs per plant.
Do		Average of 30 nymphs per plant.
Do		Average of 65 nymphs per plant.
Do		Average of 60 nymphs per plant.

[5] Consisting of commercial oleoresin of pyrethrum flowers, 9.5 ounces; oleic acid, U. S. P., 5 pounds; sodium hydroxide, C. P., 11.2 ounces; water, 2.5 gallons (*25*).
[6] Same as [5] except that a proprietary miscible oil was substituted for the oleic acid.
[7] Commercial product made according to Japanese beetle formula.
[8] Applied after plants were sprayed with water.
[9] 200-mesh dusting sulfur.
[10] Commercial product containing 70 percent of Na_2SiF_6.
[11] References to lead arsenate mean the commercial grade of acid lead arsenate.
[12] Unless stated to the contrary, all proportions of dry materials are on the basis of weight.
[13] $CS(C_6H_5NH)_2$.

OVICIDAL TESTS

Several series of tests were conducted to determine the value of some commonly used types of spray against leafhopper eggs.

Since the eggs are deposited in the plant tissue, no definite count could be made of the number treated or the number on the plant at the time of treatment. Therefore fertile females were selected and several were permitted to oviposit upon a plant for a definite number of hours. They were then transferred to another plant and permitted to remain on it for the same length of time, and this was repeated several times. Each alternate plant was then treated, leaving an intervening check for each treated plant. It was assumed that the number of eggs laid by each group of females would average approximately the same from day to day. Counts were then made to ascertain the number of eggs hatching on each treated and untreated plant. Although averages of the same treatment in different series are not fair representations of these data, owing to the use of different females for oviposition and to the different seasonal conditions, these averages are given in order to condense the detailed data. The checks showing the numbers of eggs that hatched on untreated plants exposed to the same females immediately before and after the treated plants were exposed give the best data for drawing conclusions. In table 2 the total number of eggs hatching on each treated plant, the total number of days it was exposed to females for oviposition, and the average number of females for the period exposed are used to compute the number of eggs per female per day which hatched after each treatment. For comparison, data concerning an untreated check follow the data for each treated plant.

TABLE 2.—*Tests of the ovicidal effects of materials against Empoasca fabae*

Treatment [1]	Average females on plant	Days oviposition	Total eggs hatched	Hatched eggs per day per female
	Number	*Number*	*Number*	*Number*
Nicotine sulfate [2] solution 1 pint and 2 pounds of white laundry soap to 100 gallons of water	2.0	2	25	6.2
Check	4.0	2	32	4.0
Miscible oil [3] 1 percent, resin 1–19	4.6	8	62	1.7
Check	4.1	16	186	2.8
Bordeaux mixture 3–6–50 plus arsenate of lead 1–50	4.24	9	74	1.9
Check	4.28	12	163	3.17
Bordeaux mixture 4–4–50, nicotine sulfate 1–800	4.42	11	64	1.3
Check	4.6	15	121	1.7
Miscible oil,[3] substituted for oleic acid in Japanese beetle formula [5] 1–19	3.2	8	68	2.65
Check	4.0	14	170	3.04
Summer white oil emulsion [4] 3 percent	4.5	8	73	2.03
Check	4.22	14	163	2.76
Special miscible oil [3] 3 percent, plus pyrethrum extract from 1 pound of flowers for each gallon of oil	3.0	8	43	1.79
Check	4.4	10	95	2.16
Water check	4.0	4	102	6.37
Check	4.0	4	42	2.62
Bordeaux mixture 4–6–50	5.0	2	12	1.2
Check	4.0	4	55	3.44
Pyrethrum spray (Japanese beetle formula [5] 1–15	4.75	6	20	.70
Check	4.28	11	108	2.29
Sodium oleate 1–19	4.5	4	33	1.83
Check	3.5	4	30	2.1
Average of checks	4.12	9	105	2.83

[1] Refer to table 1 for all footnotes.

Although reduced hatchings resulted from some treatments, none of these tests indicate successful control or give promise that the materials have ovicidal value, because large percentages of eggs hatched in every case. These results were verified by the fact that large numbers of eggs hatched on plots in the field treated with these same spray materials.

INSECTICIDAL TESTS

Cage Tests

Several series of insecticidal tests were performed on potted plants infested with nymphs of the leafhopper, and in the tests various types of materials were used. As indicated in table 3, which shows the resulting mortality, most of these are good insecticides. As they are not good ovicides, however, and have no effect on nymphs hatching after the application, they cannot be used economically as compared to materials containing copper sulfate, which, as shown elsewhere, are good insecticides over periods of several days and are thus effective during the period of hatching on the plant. Repeated applications must be made of these other materials for the hatching nymphs during the same period. Some of these are very toxic to the nymphs at the time of application and, as indicated, will kill all the nymphs on a plant within a few minutes' time.

Table 3.—*Cage tests with insecticides against nymphs of Empoasca fabae*

Material and strength [1]	Days after treatment	Mortality	Remarks
	Number	*Percent*	
Modified pyrethrum spray, 1 gallon of modified Japanese beetle formula [6] to 200 gallons of water 1-300.	1	91.1	Mostly killed within 1 hour after application.
Pyrethrum spray (Japanese beetle formula [5] 1-15) 1-304.	1	94.7	Mostly killed within 10 minutes after application.
Nicotine sulfate [2] solution 1 pint and 2 pounds of white laundry soap to 100 gallons of water.	1	97.7	Mostly dead 5 hours after treatment.
Commercial pyrethrum spray,[5] ½ ounce to 1 gallon of water.	1	100.0	
Special miscible oil [3] 2 percent, plus pyrethrum extract from 1 pound of flowers to each gallon of oil.	1	100.0	Killed within 5 minutes after treatment.
Special miscible oil [3] 1 percent, plus pyrethrum extract from 1 pound of flowers to each gallon of oil.	1	100.0	
Special miscible oil [3] 0.5 percent, plus pyrethrum extract from 1 pound of flowers to each gallon of oil.	1	100.0	30 dead in ½ hour.
Sodium oleate:			
1-19	1	100.0	
1-76	1	69.5	
1-152	1	70.0	
Bordeaux mixture 3-6-50 plus arsenate of lead 1-50	1	70.0	
Do	2	90.0	
Bordeaux mixture 3-8-50	1	56.8	
Do	2	88.6	
Bordeaux mixture 6-6-50	1	47.6	
Do	2	80.9	

[1] Refer to table 1 for all footnotes.

The results of these tests would indicate that pyrethrum, nicotine, and specially made oil emulsions are the best control materials; but, as indicated under the discussion of the field-test plots, certain of these do not give similar results under field conditions and very few, if any, have any effect on nymphs hatching later. Therefore percentages of mortality obtained in cage tests tell only a portion of the story.

After these field studies had revealed that bordeaux mixture had a residual value which caused newly hatching leafhoppers to be killed soon after feeding on a treated plant, it was decided to make a special study of bordeaux mixture as an insecticide, and especially to find under what conditions and in what ways it caused insect toxicity.

BORDEAUX MIXTURE AS AN INSECTICIDE

Resume of the History of Bordeaux Mixture and Previous Work Performed

Extensive investigations of bordeaux mixture have been made, principally by botanists, plant pathologists or physiologists, and chemists. Before attempting to discuss the present investigations and conclusions drawn from them, therefore, it will perhaps be well to survey in brief the history of bordeaux mixture and the more important and recent work performed on it by various workers, especially in the bearing on the problem at hand.

So far as records go, the first recognition of bordeaux mixture as a fungicide dates back to the fall of 1882. It was then that Millardet (*37*) discovered that a mixture of copper sulfate and lime was an excellent control measure for downy mildew of the grape. This compound of copper sulfate and lime soon became known as bouillie bordelaise, or bordeaux mixture, and was used extensively as a fungicide after 1887, when Millardet and Gayon (*38, p. 704*), after extensive experimentation, improved and recommended the mixture for general use.

Later bordeaux mixture was found to possess both repellent and insecticidal properties, especially when ingested with food. An attempt was then made to increase its general effectiveness as a pest control by the addition of arsenicals and other inorganic compounds, as stomach poisons for chewing insects, and organic poisons like nicotine. Bordeaux mixture alone, however, was not considered as an effective control for insects, especially those with piercing, sucking mouth parts. Bourcart, as mentioned later (*6*), was the only one to report otherwise concerning its effect upon piercing, sucking insects until 1922.

The most perplexing problems in the use of bordeaux mixture have related to its action on plants or plant processes, and even the question of its use and value as an insecticide has involved this same problem. There is still a great diversity of opinion as to the exact manner in which the copper sulfate in bordeaux mixture can function as a fungicide and at the same time exert a stimulative action on plants and be toxic to insects.

It has been thoroughly demonstrated that the supernatant liquid of properly prepared bordeaux mixture contains only very minute quantities of dissolved copper, and these require very delicate tests for recognition. When plants are sprayed with bordeaux mixture the residue left on the foliage after the spray has dried contains copper compounds of very small solubility in water. The precipitate formed in the preparation of bordeaux mixture was at first believed to be copper hydroxide. According to Pickering (*41*)—

> The substances formed on the addition of lime to copper sulphate as in the preparation of Bordeaux mixture are dependent on the proportions of lime used, and may be either (approximately so far as the $CaSO_4$ is concerned): (1) $4CuO, SO_3, 0.06\ CaSO_4$, (2) $5\ CuO, SO_3, 0.25\ CaSO_4$, (3) $10\ CuO, SO_3, 1.30\ CaSO_4$, (4) $10\ CuO, SO_3, 4CaO, SO_3$, (possibly 5) $10\ CuO, SO_3, 10\ CaO, SO_3$, or (6) $CuO, 3CaO$; that present in most cases probably being (4).

The basic sulfates $4CuO \cdot SO_3$ and $5CuO \cdot SO_3$ are somewhat soluble in the mother liquor, the former 1 part in 40,000 and the latter only 1 or 2 parts per million. The other basic salts are quite insoluble in the mother liquor, but these dissolve to a slight degree in solutions of dextrose, saccharose, and other similar organic substances.

The composition of bordeaux mixture will vary in the number and proportion of these basic sulfates, depending on the ratio of copper sulfate to the calcium hydroxide used. According to Butler (*7, p. 150*), if a moderately strong lime is used with copper sulfate in proportions of 1:1 or stronger, a more voluminous basic sulfate tends to form composed of the higher basic sulfates ($10CuO \cdot SO_3$ and $10CuO \cdot SO_3 \cdot 3CaO$).

THE SOLUBILITY OF COPPER FROM BORDEAUX MIXTURE

Since the effect of bordeaux mixture on insects is apparently through the plant, the conditions determining its effectiveness are of basic consideration in a study of bordeaux mixture as an insecticide. In 1909 Crandall (*14, pp. 223–228*) summarized the work that had previously been completed on this subject and discussed at length the question of the solvent action of carbon dioxide meteoric waters, ammonium compounds, nitrates, nitrites, and other materials upon the copper from bordeaux mixture. Millardet and Gayon (*38, p. 699*) emphasized the effectiveness of carbon dioxide as a solvent for copper from bordeaux mixture. Swingle (*52, p. 20*) suggests the possibility that rain water and dew upon the plant leaf absorb sugar and other substances that may increase the solubility of copper. Clark (*10, p. 42*) found that the host plant, when placed in water, is responsible for the solubility of copper from bordeaux mixture. He further describes a condition which he calls exosmosis. Bain (*4, pp. 53–54*) studied especially the possibility of glandular secretions on the leaf and petiole causing the solubility of copper. According to the view of Schander (*44, p. 526*), germinating spores are responsible for the solution of copper from bordeaux residue on the leaves. He designates the following three possibilities regarding the action of bordeaux mixture on plants: (1) That acid secreted by the plant dissolves copper from copper hydroxide, the leaves then taking up this dissolved copper and being injured (*44, p. 579*); (2) that glandular secretions may be alkaline but act as solvents for copper; and (3) that small quantities of copper salts are dissolved by rain and dew and penetrate the epidermis into the interior of the leaf (*44, p. 582*). According to Ostwald (*40*) certain complex copper compounds are formed when lime is added in excess to copper salts in the presence of organic compounds like sugar, which are not precipitated from solution by alkalies.

THE PENETRATION OF COPPER INTO LEAF TISSUES

Authorities also disagree considerably as to how copper, if dissolved from bordeaux residue, can penetrate into plant tissues and exert its known effects on plants without injuring them. This problem is of fundamental importance also in relation to the insecticidal problem.

When water-soluble copper enters the leaf it frequently causes a toxic influence by killing the protoplasm of a number of cells in a limited area. Millardet and Gayon (*38*), Aderhold (*1*), Schander (*44, p. 536*), and others hold the view that copper in solution penetrates the cells by direct contact. According to this theory the copper is locked in the dead cells and does not spread through the leaf. On the other hand Rumm (*42, 43*) proposed a theory which he calls chemotactic action, and he suggests an electrical attraction between cell protoplasm and the bordeaux film upon the leaf. Nägeli (*39*) found that the chlorophyll bands of *Spirogyra* were broken up by the proximity of metallic copper, and he named the force which caused this action oligodynamic. Rumm's theory of poisonous action resulting from contact without penetration was based on his work on *Spirogyra* and was supported by Frank and Krüger (*28*) and by Zucker (*54*).

Crandall (*14, p. 229*) quoted [by translation] from Schander (*44, p. 539*):

> Zucker found plants treated with Bordeaux more resistant to etiolation than untreated plants, and partially etiolated plants placed under treatment developed the normal green color. He regards the action as an electrical stimulus and "since in all plants more or less weak electrical currents are produced by the motion of the water in the capillary spaces, it is reasonable that the deposition of a strongly electro-positive substance, like copper hydroxide, upon the leaves should be capable of intensifying the plant currents with stimulating effect upon the activity of the protoplasm.

According to Aderhold (*1*) and Schander (*44, p. 537*) the stimulating effect of copper is caused only rarely and then by penetration of the cells, whereas injury is attributed to the accumulation of copper in sufficient quantity to kill cells.

Schander (*44, p. 545*) conducted experiments in which pricked and uninjured leaves were exposed to copper sulfate solutions. Injured leaves were penetrated by weak copper solutions. He concluded that the epidermis of normal leaves was capable of preventing the penetration of copper compounds. If penetration and acumulation of copper does occur, it probably is present only in minute quantities unless chemical changes take place which form new compounds that are noninjurious to living cell protoplasm.

EFFECT OF BORDEAUX SPRAYS UPON THE PLANT

Various phases of this subject have been studied by several workers, A brief summary of their results and conclusions is sufficient to show that most of them believe that bordeaux sprays cause changes in either the morphology or the physiology of the plant or both.

Copper salts, according to Sorauer (*49, p. 33*), caused the parenchyma cells to be hypertrophied.

Frank and Krüger (*28*) secured a better growth of bordeaux-sprayed potatoes and they considered the effect more physiological than morphological. A rise in transpiration occurred. The chlorophyll content was apparently increased and more starch was formed. The leaves were longer and thicker and the length of life was greater when bordeaux mixture was applied. The greater quantity of starch produced required storage and this took place in the tubers. They found the ratio between tubers produced on treated and untreated plants to be 19:17 and 17:16. They concluded that an increase in photosynthesis resulted due to the copper sprays.

Rumm (*42, pp. 83–85*) found that bordeaux-sprayed leaves were darker in color and more robust than those unsprayed. Microscopic examination showed an increase in the number of chlorophyll grains in the sprayed leaves, and measurements of the thickness of sprayed and unsprayed grape leaves gave a range of 2.17 to 16.31 micromillimeters greater in favor of the sprayed leaves. Furthermore, he (*43*) thought that morphologic changes took place due to copper which penetrated the growing leaf. The parenchyma, the palisade cells, and the epidermis were all thickened on the sprayed plants.

Lodeman (*33*) also obtained a thickening of both plum and prune leaves by similar applications. Harrison (*30*) produced thickened leaves also on plum, peach, and pear with bordeaux sprays. Zucker (*54*), working entirely in the greenhouse, obtained increased transpiration and an increase in the chlorophyll content and the assimilative power by the use of bordeaux sprays. Chuard and Porchet (*9*) obtained the same results by injecting dilute solutions of copper into the plant that others had obtained by spraying the plant with bordeaux mixture. By injecting copper into grape they obtained more intense color and more vigorous growth and increased the length of life of the leaves.

Schander (*44, p. 576*) thought that the shading effect of bordeaux residue on the leaf caused a greater absorption of carbon dioxide. He believed also that copper which penetrated the leaf changed the rate of respiration and assimilation. Ewert (*22*) concluded that bordeaux mixture hindered rather than stimulated plant growth because absorbed copper checks the diastase action and the starch is piled up in the chlorophyll bodies. Amos (*2*) found that bordeaux mixture diminished the assimilation of the leaves for a short time after treatment by blocking the stomata of the leaf, causing less air to diffuse into the intercellular spaces.

Dugger and Cooley (*20*) used potted plants and showed that the rate of transpiration was increased in potato plants treated with bordeaux and other films. Lutman (*35*) in his early work concluded that small quantities of copper enter the leaf and that a chemical combination takes place between the copper and the chlorophyll. He also concluded that the palisade and parenchyma cells were thickened by the use of bordeaux mixture and that the number of chlorophyll bodies was increased. In his later work he decided that the physiological effect was due to reduced tipburn and flea beetle injury and not to stimulation.

EARLY NOTICE OF TOXIC EFFECTS ON INSECTS

The larger part of the work on bordeaux mixture as an insecticide reported to date has been performed within the last few years. A few early workers, however, reported successful control with this material.

Bourcart (*6, p. 260*) noted:

> Bouillie bordelaise is regarded by Dr. Menudier, Mohr, and D'Angelo as being sufficient to remove the phylloxera. D'Angelo has used this process in the island of Elba with full success. The treatment comprised two sprayings of the stock with a bouillie bordelaise of 1.8 per cent blue vitriol and 1 per cent lime, and three sprayings with a 5 per cent solution of blue vitriol containing sulphur.

This control was in all probability attributed to either a repellent or a contact insecticidal action. The addition of sulfur to certain of these spray treatments would tend to substantiate this view.

In discussing other experiments performed by D'Angelo, Bourcart (*6, p. 211*) states that he—

> met with complete success against the phylloxera in the island of Elba, by injecting blue vitriol into the diseased stocks. Dying vines revived by this treatment, for the phylloxera disappeared from the stocks treated.

Apparently two things may have happened. The disappearance of the phylloxera was apparently due to a host therapeutic condition, for it is known today that when some plants are treated with copper compounds, especially bordeaux mixture, the sap becomes toxic to certain sucking insects. Likewise the dying plants were probably revived more quickly because, as it is thought at the present time, copper salts, especially copper sulfate, when applied in proper proportions, have a stimulating effect upon plants. It is apparent that D'Angelo did not recognize what he had accomplished, or the importance of his observation. Otherwise, it would not have been necessary to wait until 1919 for further insecticidal observations when Fluke (*26*) and, later, Dudley and Wilson (*19*) and Fenton and Hartzell (*23, 24*) proved that bordeaux mixture alone was toxic to the potato leafhopper (*Empoasca fabae*) an insect with piercing, sucking mouth-parts.

The investigators last mentioned noticed that leafhoppers had disappeared from plants that had been treated with bordeaux mixture a few days previously, and attributed this in part to a repellant action of the spray. They did, however, remove single leaves from treated plants and enclose them in shell vials with individual leafhoppers. While the condition of the leaves and leafhoppers left thus enclosed would not definitely explain in any way how they were affected by bordeaux mixture, the observations made should be mentioned here. They found that in not a single case did any of the nymphs feeding on sprayed leaves become adults. They also noted that the nymphs "would moult once but very often death occurred directly after the casting of the exuvium or even before the nymph could completely extricate itself"; also they state that "the younger the insect, the quicker death resulted upon being confined on a sprayed leaf, the length of time ranging from approximately two days in the case of the first instar to about six for the fifth." On unsprayed leaves the nymphs could easily be reared to maturity in similar vials. On the other hand they were in doubt regarding the toxicity of bordeaux mixture against adult leafhoppers but thought it not very toxic.

With this preliminary work and these observations on this compound as a beginning, the writer, with others, began an investigation in 1928 to determine if possible the role of bordeaux mixture as an insecticide (*15, 17, 18*).

Observations were first made in the field upon several series of bean plants that had been treated with a variety of materials, including bordeaux mixture. In these plot tests it was noted that pyrethrum extracts killed all the leafhoppers within a few hours after the spraying. They had no effect, however, on the young leafhoppers that hatched a few hours after treatment, consequently those able to hatch during a period of about 10 days after treatment, from eggs already placed in the plant tissue, soon comprised a rather large population. In the experiments with bordeaux mixture it was observed that there was no immediate effect on the leafhoppers, for at the end of 24 hours after spraying they were as abundant as at the time of application. After this period, however, they died gradually, until at the end of 3 or 4 days all had succumbed. Observation a week after spraying showed no new leafhopper populations, and the newly hatched nymphs, except the very small ones, were dead. The most interesting phases of this control of the leafhopper by bordeaux mixtures seemed to be a delayed but certain killing of the individuals present and a continued lethal action against hatching nymphs for a considerable time after application.

It is a common field practice in leafhopper control to spray the crop when the insect is present, the spray thus coming in contact with the leafhopper. This type of treatment was used on potted plants as a starting point in the experiments to test the action of bordeaux mixture as an insecticide. Plants thus treated showed some nymphs of all stages dead 24 hours after spraying, and at the end of 4 days not more than 5 percent of the total leafhopper population survived. At the end of 6 days after treatment all the leafhoppers usually were dead. At the end of the same 6-day period leafhopper checks upon untreated plants under conditions exactly the same as those upon treated plants showed 85-percent survival. The leafhoppers apparently feed as normally on the treated plants as they do on untreated plants and, furthermore, there is no indication that they attempt to leave the sprayed plants for unsprayed plants.

Tests to Determine Whether Bordeaux Mixture Is a Contact Insecticide

In view of results obtained by spraying plants when the leafhoppers were on them, experiments were performed to determine, if possible, whether bordeaux mixture kills by contact with the insect. The problem was approached in two ways in order to determine what had been the action of bordeaux mixture on the leafhoppers in the series of experiments just discussed. The first method consisted of spraying the plants and then placing the leafhoppers upon them after tho bordeaux mixture had dried, thus eliminating the possibility of the spray covering the insects. The second method was to drench the leafhoppers thoroughly with the bordeaux mixture and then place them upon untreated plants, thereby ascertaining whether this material has any value as a direct contact insecticide.

Both nymphs and adults in large numbers were tested by the first method. The interval of time after treatment and before the insects were placed on the plant was varied greatly in different trials. When leafhoppers were placed on these plants a few hours after treatment, all died within 4 or 5 days. If not placed upon the treated plants until 2 to 3 weeks after the spraying, the leafhoppers died in approximately the same time as they did when placed on the plants soon after the plants were sprayed. This indicated that it was not necessary to strike the insects with spray material to kill them.

There still remained the possibility that the residue of bordeaux mixture, when dry upon the plant, might act as a contact insecticide as the leafhoppers moved about on the leaf. To eliminate such possible action an attempt was made first to remove the residue from treated plants by washing them in running water a few days after they were sprayed. The leaves were cleansed as thoroughly as possible without mutilating the tissue. Although all visible stains and residue of bordeaux mixture were removed from these plants, the leafhoppers placed upon them died in 4 or 5 days. Typical symptoms indicated that the insects were being killed by effects of the bordeaux mixture. The period of time required to kill the insect corresponded in these experiments to the cases where the plant foliage was normally still covered with the spray while the leafhoppers were being permitted to feed upon it.

The second method was to spray the leafhoppers and then place them on untreated plants. Leafhopper nymphs were sprayed in a container in order to effect a thorough drenching and it was sometimes necessary to rescue many of them from a pool of bordeaux mixture. Many of these were so completely soaked that they were not active for several minutes after treatment. When they showed signs of activity, the leafhoppers were immediately placed upon untreated plants, where they fed normally during the remaining days they were under observation. In spite of this severe treatment, less than 5 percent had died at the end of 6 days, and in certain specific tests all the leafhoppers survived such contact treatments and remained alive from 10 to 12 days.

EXPERIMENTS WITH FEEDING CELLS

An attempt was next made to eliminate the contact factor entirely. In view of the negative results obtained from the experiment performed to determine the value of bordeaux mixture as a contact insecticide, and in the light of previous results by other workers on the effect of bordeaux mixture as a stimulating agent to plant tissues, a series of experiments were carried on to determine the effect of bordeaux mixture on leafhoppers when they were permitted to feed on bordeaux-sprayed plants but were prevented from coming in contact with the spray material on the plant. This was accomplished by preventing the portions of foliage upon which the insects were to be fed from being hit by spray material. It was also necessary to enclose the insects in a specially constructed feeding cage so as to confine their feeding to the unsprayed part of the foliage in order to be certain that the contact factor was not causing the results obtained. In these experiments only one surface of the leaf was treated and the leafhoppers were confined to the other surface.

Different types of glass feeding cells were tested and the best results were obtained with pieces of glass tubing about 2½ inches long and 2½ inches in diameter with a ring of chamois cloth a little wider than the thickness of the glass wall glued on the edge of the glass at one end. The cloth, when brought in contact with the leaf surface, makes a closely fitting joint between the lower surface of the leaf and the hairy fiber of the cloth, preventing the leafhoppers from escaping between the edge of the glass where, because of the large veins in the undersurface of the leaf, it does not touch the leaf tissue. The other end of the glass cell was covered with thin cheesecloth to allow free circulation of air. The cells were pressed against the selected leaves and held at the proper height by wire supports stuck into the ground (fig. 1). In this type of cell the leafhopper can feed normally under controlled conditions.

As a rule the upper surface was treated and the leafhopper nymphs were placed upon the undersurface, since this is the normal position of feeding under field conditions. It was soon observed that a small number of nymphs took all the cell sap from a small unit of leaf tissue in a very short time. Consequently a considerable area of leaf tissue was necessary for the continued feeding of only a small number of leafhoppers while they were under observation. The plant juices are apparently extracted and pumped through the bodies of the leafhoppers in large quantities, as evidenced by the large quantity of excrement deposited on the walls of the feeding cells. It was necessary, therefore, to limit the number of leafhoppers in a single cell to five or six at a time and to use a number of cells in order to have a representative number of insects in each experiment. Glass feeding cells of the same type were used as checks on untreated plants in

FIGURE 1.—Glass feeding cells for experiments with leafhoppers.

exactly the same way and with the same number of leafhoppers to a cell. The results of these tests were variable owing to differences in temperature, size of nymphs, and other factors, but in general they told the same story in every case.

In one series of tests 60 percent of the leafhoppers had died 5 days after they had been placed on the plants, and less than 5 percent of those in the check had died. At the end of 10 days the treated plants showed 82 percent dead and the check less than 5 percent. Another series showed 84 percent dead on the treated plants at the end of 7 days and 50 percent dead on the checks, and at the end of 14 days 95 percent dead on the treated and 50 percent dead on the checks. Although the check in this case showed a high mortality, the mortality was almost twice as great on the treated plants. The presence of a natural enemy, too, had apparently contributed to the death of some of the leafhoppers on the check plants. These enemies were not present on the treated plants.

A third series was performed when temperatures were much lower and when the rates of feeding and metabolism were consequently much slower. Under these conditions only 50 percent were dead in 10 days and only 62 percent in 14 days, whereas the check showed 18 percent dead at each examination.

Although the mortality varied greatly in these different series of experiments, the bordeaux-sprayed plants in each case showed a much higher mortality than the check plants. It will be noted also that the rate of killing was slower in all the experiments where cells were used than where the insects were placed on plants treated with bordeaux mixture on both surfaces of the leaves. Since only one surface of the leaf was sprayed, however, the quantity of bordeaux mixture present on the leaf was only about one-half as great as in these other tests and consequently the amount of copper available must have been correspondingly less.

While the plants were being sprayed the roots were sufficiently protected to prevent any bordeaux mixture from getting into the soil or coming in contact with the roots. Under field conditions it is entirely possible that the roots might absorb bordeaux mixture, but the spray on the leaves would seem to be the more important factor.

While these tests have not proved the point in question, they have given strong indications that these leafhoppers obtained copper in some form, or some plant product induced by this spray, from the leaf tissue upon which they fed, and were apparently obtaining this material from the cell sap. It is known that when bordeaux mixture is sprayed upon the leaves it apparently stimulates plant growth in some cases, and that other insecticides in the soluble form are taken up by the plants (*13, 52, 53*), and the indication from these leafhopper tests is in accordance with this known effect upon the plant. Smith and Poos (*48*) have recently demonstrated by microscopic examination of plant tissues injured by *Empoasca fabae* that during exploratory punctures made by this leafhopper while in search of fresh phloem the mouth parts sometimes reach the epidermis on the opposite side of the leaf.

Symptoms of Bordeaux Poisoning

Observations made during these experiments have indicated that certain conditions or symptoms are characteristic in leafhoppers that die from the effects of feeding on foliage sprayed with bordeaux mixture. In these cases the characteristic bright-green color is usually replaced by yellow within 48 hours after they have begun to feed. In 2 or 3 days after beginning to feed on bordeaux-sprayed foliage they become weakened or inactive and fall from the plants. After once dropping from the plant they are unable to get back or move about in any decided way, and if placed on the plant they are not able to remain upon it. This seems to be some type of motor paralysis, and the insects eventually lose their ability to move about.

This condition seems linked more or less with the most characteristic feature of poisoning from bordeaux mixture, that is, the inability of the insect to molt successfully. A large proportion of poisoned insects die during the molting process. This fact was also noted by Fenton and Hartzell (*24*). The process of molting begins as usual and the anterior part of the body is normally freed from the cast skin, but the leafhopper is unable to free the last pair of legs and the posterior portion of the abdomen and dies in this partly molted condition. In the emerging adult the wings are still curled and the body is frequently strongly arched at the middle and remains this way until death occurs. If carefully freed from the partly molted skins, the leafhoppers do not live longer than if not disturbed. This seems to be a case of death due to a weakened condition, although it is possible that the molting fluids are affected chemically, especially since the cast skins sometimes show a light copper-blue color instead of the normal white.

Size of Nymphs as Related to Toxicity

Small nymphs are more quickly killed than those in the fourth or fifth instars. If only first-, second-, and third-instar nymphs had been used in all these tests, not only would the length of time for killing have been reduced, but the percentages killed would have been greater, as indicated by the higher percentages of small nymphs killed. Many of the large nymphs successfully molted to the adult stage before death, which occurred from 1 to 10 days afterward in some cases. As a rule a large nymph cannot molt successfully to the adult stage if it has been longer than 48 hours on a treated plant. Where experiments were carried on with adults a much smaller percentage was killed and a longer time was required for killing.

Toxicity of the Plant Sap

Certain facts are evident from the experiments just reported. Leafhoppers, which have piercing, sucking mouth parts and feed exclusively on plant sap secured from beneath the epidermal cells, were killed by covering the host plant with the residue of a spray that had no appreciable effect on the leafhoppers when it was used as a contact insecticide. This suggested very strongly that the insects were securing a poison from the plant sap and that the ultimate action was a stomach poison. There was a slight possibility that the leafhoppers could ingest a sufficient quantity of the bordeaux residue coating the epidermis of the plant to be poisoned thereby. It is well known that leafhoppers and other similar sucking insects can feed on plants having equivalent residual coverings of materials such as the arsenicals, which are much more toxic as stomach poisons than bordeaux mixture, without being affected. All the evidence thus pointed to the conclusion that the sap of the plant treated with bordeaux mixture became toxic. The leafhoppers that were confined to the unsprayed surface of a sprayed leaf and yet showed high mortality added evidence to the theory that the insecticidal action of bordeaux mixture was a problem of host therapy which probably resulted from the residue of bordeaux mixture left on the plant after the spray had dried.

It is evident that an investigation into the problem of how bordeaux mixture, when sprayed on bean and potato plants in an insoluble form, is able to render these plants toxic to the potato leafhopper, a sucking insect, involves chiefly two major possiblities regarding the action of bordeaux mixture on plants, (1) that copper is dissolved from the bordeaux mixture, and (2) that this dissolved copper penetrates into the plant tissue.

If there is a possibility of the plant absorbing copper and if this copper can poison the leafhoppers, then aqueous solutions of copper compounds should be toxic when ingested directly by these insects. Tests were therefore conducted to find if possible whether copper or calcium salts in various dilutions were toxic to the potato leafhopper.

EXPERIMENTS WITH MEMBRANE FEEDING CELLS

Carter (*8*) has demonstrated by his work with the beet leafhopper (*Eutettix tenellus* (Baker)) that leafhoppers will feed on certain solutions through thin membranes. For these tests about to be discussed,

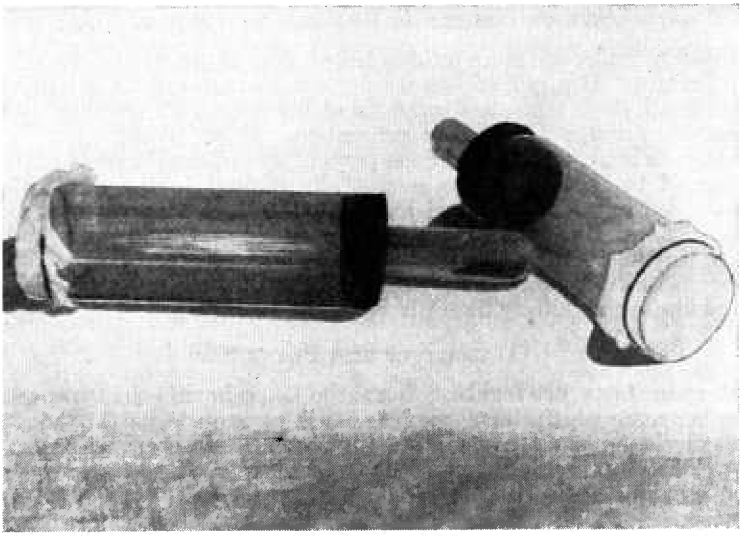

FIGURE 2.—Cells used for experiments in the feeding of copper solutions through membranes.

membrane feeding cells (fig. 2) were constructed, each cell consisting of a piece of glass tubing 5 inches long and 1½ inches in diameter. One end of the tube was fitted with a cork stopper bored to accommodate the open end of a glass vial 3 inches long by eleven-sixteenths of an inch in diameter. The vials each held approximately 20 cc. of liquid. The solution to be fed was placed in the vial and the open end was then covered with a capping skin membrane. The membrane-covered end was then inserted in the cork stopper until the membrane was flush with the inner surface of the cork. A sufficient margin was left on the membrane, and the vial fitted so snugly into the cork that the membrane was held tightly over the end and was leakproof when inverted. The other end of the tube was covered with a piece of cheesecloth in an attempt to keep the temperature and humidity as nearly normal as possible.

Usually 10 leafhopper nymphs were put in each feeding cell for each series of tests. The solutions put in these vials apparently did not attract the leafhoppers, but by placing these feeding cylinders so that the source of light came through the membrane, the leafhoppers would move immediately to this end and soon attempt to feed by puncturing the membrane with the mouth parts. After they had become accustomed to feeding in this way, they would readily return to the membrane after having been disturbed and would remain for some time without moving when feeding.

It was thought desirable to ascertain the action of different food solutions containing copper and calcium salts at different dilutions, as well as the effect of copper and calcium salts alone at various concentrations on both nymphs and adult leafhoppers. This should give both the comparative toxicity of the two materials and the relative toxicity of the various dilutions of either material. Only data obtained with nymphs are included.

Using the same method of experimental feeding, it had been determined previously that leafhopper nymphs of the second, third, and fourth instars lived on a 5-percent solution of cane sugar for an average of 12 days with a maximum survival period of 32 days. When permitted to feed on distilled or tap water alone, the average period of survival was 3 days and a maximum of 5 days. Such a test might be considered a starvation-point test, since very little if any food is obtained from distilled water. It was quite evident that when leafhoppers were feeding on solutions of copper sulfate and died in about 3 days' time, the cause of death might have been starvation. Therefore a 5-percent cane-sugar solution was used with each dilution of copper sulfate in certain series of tests, provision being thus made against death by starvation. The relative toxicity of dilutions of copper with sugar and without sugar are shown in table 4, with the maximum, minimum, and average survival for each dilution. These data are based on approximately 2,600 individual records used in replicated tests averaging 8 individuals per test.

TABLE 4.—*Feeding experiments with leafhopper nymphs in membrane feeding cells*

GROUP 1. GIVEN COPPER SULFATE ($CuSO_4 \cdot 5H_2O$) IN THE SOLUTIONS INDICATED[1]

Food solution used and concentration	Survival period			Food solution used and concentration	Survival period		
	Maximum	Minimum	Average		Maximum	Minimum	Average
	Days	*Days*	*Days*		*Days*	*Days*	*Days*
1-100	1.7	0.25	0.8	1-3,000	12.0	1.0	3.2
1-300	1.7	.75	.9	1-3,332	5.7	1.0	2.8
1-500	4.0	.75	1.28	1-4,000	19.0	1.0	3.59
1-666	1.5	.9	1.1	1-5,000	27.0	1.1	4.28
1-700	4.0	.75	2.25	1-6,000	20.0	1.3	7.2
1-900	5.0	1.0	2.25	1-6,666	10.0	1.0	3.9
1-1,000	5.0	.7	1.54	1-7,000	29.0	7.0	12.8
1-1,500	5.0	.75	2.43	1-7,500	13.0	.75	3.27
1-2,000	6.8	.75	2.92	1-8,000	19.0	3.0	10.9
1-2,500	8.0	.75	2.97	1-9,000	28.0	4.0	10.4
1-2,666	4.6	1.3	3.1	1-10,000	14.0	.75	5.04

GROUP 2. CHECK ON GROUP 1.

Sucrose, 5-percent solution	32.0	0.8	10.3	Tap water	7.0	0.8	2.92
Distilled water	7.0	.8	2.4	Dextrose, 5-percent solution	9.0	.8	3.3

[1] 1 gm. of the salt to the indicated number of cubic centimeters of a 5-percent sucrose solution.

TABLE 4.—*Feeding experiments with leafhopper nymphs in membrane feeding cells*—Continued

GROUP 3. GIVEN CALCIUM SALTS IN THE SOLUTIONS INDICATED [1]

Food solution used and concentration	Survival period			Food solution used and concentration	Survival period		
	Maximum	Minimum	Average		Maximum	Minimum	Average
	Days	*Days*	*Days*		*Days*	*Days*	*Days*
$CaCl_2$ [2] $.H_2O$:				$Ca(NO_3$ [3]$)_2.4H_2O$:			
1-100	3.0	0.8	1.1	1-100	12.0	0.8	2.4
1-500	13.0	1.5	5.7	1-500	22.0	1.0	12.5
1-1,000	23.0	2.0	13.0	1-1,000	25.0	1.0	15.2

GROUP 4. GIVEN COPPER SULFATE ALONE IN THE PROPORTIONS SHOWN [4]

1-100	1.5	0.75	0.8	1-4,000	4.0	1.00	2.9
1-300	1.5	.75	.87	1-5,000	7.0	2.00	3.4
1-500	1.5	.75	1.02	1-6,000	4.0	2.00	3.2
1-700	3.0	.75	1.25	1-7,000	4.0	1.00	2.9
1-900	3.0	1.00	1.95	1-8,000	4.0	1.00	3.0
1-1,000	4.0	.75	1.97	1-9,000	5.0	3.00	2.9
1-2,000	4.0	1.00	2.7	1-10,000	5.0	3.00	3.4
1-3,000	4.0	2.00	3.1				

GROUP 5. GIVEN COPPER SULFATE IN THE SOLUTIONS INDICATED [1]

1-66	1.0	>1.0	1.0	1-2,666	10.5	>1.0	3.6
1-666	1.0	>1.0	1.0	1-3,332	14.0	>1.0	6.9
1-1,666	1.0	>1.0	1.0	1-6,666	22.0	11.0	15.4

GROUP 6. GIVEN COPPER SULFATE IN SOLUTIONS INDICATED [5]

1-666	>1.0	>1.0	>1.0	1-2,666	0.5	1.0	4.1

GROUP 7. GIVEN CALCIUM SALTS AS INDICATED [1]

$Ca(OH)_2$:				$Ca(NO_3)_2.4H_2O$:			
1-100	1.0	>1.0	>1.0	1-100	14.0	>1.0	8.0
1-250	8.0	>1.0	1.3	1-1,000	23.5	2.5	15.9
1-1,000	30.0	>1.0	15.8				
$CaCl_2.H_2O$:							
1-100	3.0	>1.0	1.2				
1-1,000	20.5	11.0	16.3				

GROUP 8. CHECK LOT.

5-percent sugar	30.0	4.0	15.5	10-percent sugar	17.0	8.0	12.6
Distilled water	9.0	>1.0	2.1				

GROUP 9. GIVEN THE SUPERNATANT LIQUID FROM BORDEAUX MIXTURE

Bordeaux mixture 4-4-50 freshly made	3.0	1.0	1.9	Bordeaux supernatant fluid (96 hours settling) [6]	3.0	0.7	1.6
Bordeaux supernatant fluid (2 hours settling) [6]	3.0	1.0	2.1	Bordeaux supernatant fluid (2 hours) + 5 percent sugar	30.0	4.0	14.6
Bordeaux supernatant fluid (24 hours settling) [6]	4.7	2.0	2.5	Bordeaux supernatant fluid (96 hours) + 5 percent sugar	26.0	1.7	5.1

[1] 1 gm. of the salt to the indicated number of cubic centimeters of a 5-percent sucrose solution.
[2] Commercial grade of $CaCl_2.H_2O$ used.
[3] Commercial grade of $Ca(NO_3)_2.4H_2O$ used.
[4] 1 gm. of the copper salt to the indicated number of cubic centimeters of water; no sugar.
[5] 1 gm. of the copper salt to the indicated number of cubic centimeters of a 10-percent sucrose solution.
[6] No copper shown by test with H_2S.

Although calcium salts have not been tested so extensively as copper sulfate, preliminary tests are shown in table 4. From a study of this table it can be seen that copper sulfate is much more toxic than the calcium salts to leafhopper adults. It seems to be 10 times as toxic. Calcium salts used at a 1–1,000 dilution gave a survival equal to that of the 5-percent sucrose, while only copper-sugar solutions more dilute than 1–10,000 gave similar and consistent results.

Leafhopper nymphs feeding upon these solutions exhibited what has previously been termed characteristic symptoms of bordeaux poisoning. They died in faulty ecdysis and frequently with the back strongly arched and the partly cast skins distinctly bluish.

Toxicity of Copper in Bordeaux Mixture Solution

When properly prepared the basic copper sulfate in bordeaux mixture is thought to be practically insoluble in the mother liquor. In such a case the clear supernatant liquid should not be toxic to leafhoppers when they are permitted to feed on it. In order to test this toxicity, standard bordeaux mixture 4–6–50 was prepared and allowed to settle for about 2 hours. The supernatant liquid was then siphoned off and converted into a 5-percent sugar solution by the addition of the proper quantity of sugar crystals. Leafhoppers permitted to feed on this solution survived, on an average, 14 days. The supernatant liquid was also tested for copper and gave no evidence of copper where the reaction would have showed the presence of copper, if there, equivalent to a 1–50,000 solution of crystalline copper sulfate. There is certainly nothing in this solution that is toxic to the nymphs, and these tests have verified the opinion that the copper in bordeaux precipitate or residue is practically insoluble in the supernatant liquid.

Toxicity of Copper to Leafhoppers When it is Obtained Only Through the Plant Tissue

An attempt was made to test the toxicity of copper when the insect could obtain it only through the medium of the plant sap. The roots of bean plants were placed in solutions of copper sulfate of different concentrations (fig. 3), and the leafhopper nymphs were placed on the plants immediately after the roots had been placed in these solutions.

The results varied somewhat, because of the slight variation in size or age of the nymphs. The most clear-cut and striking results were obtained with a 1–100 dilution of copper sulfate. Leafhoppers feeding at an average temperature of approximately 80° F. upon plants whose roots were placed in this solution died within 6 hours, though the plant showed no signs of the effects of copper. At lower average temperatures (approximately 70°) 97 percent of all leafhoppers on these plants died within 48 hours.

Ninety percent of the leafhoppers died in 3 days when feeding on plants whose roots were placed in a 1-200 solution, 100 percent died in 3 days on a 1–250 solution, 96 percent died in 5 days on a 1–300 solution, 69 percent died in 5 days on a 1-500 solution, 75 percent died in 5 days on a 1–900 solution, 62 percent died in 5 days on a 1–900 solution, and 62 percent died in 5 days on a 1–1,000 solution.

These data show that a marked toxicity occurred within 3 to 5 days for all dilutions up to and including a 1–1,000 solution of copper sulfate.

Strengths of from 1–1,000 to 1–7,500 gave from 80 to 40 percent mortality, respectively, in approximately 10 days, whereas the plants

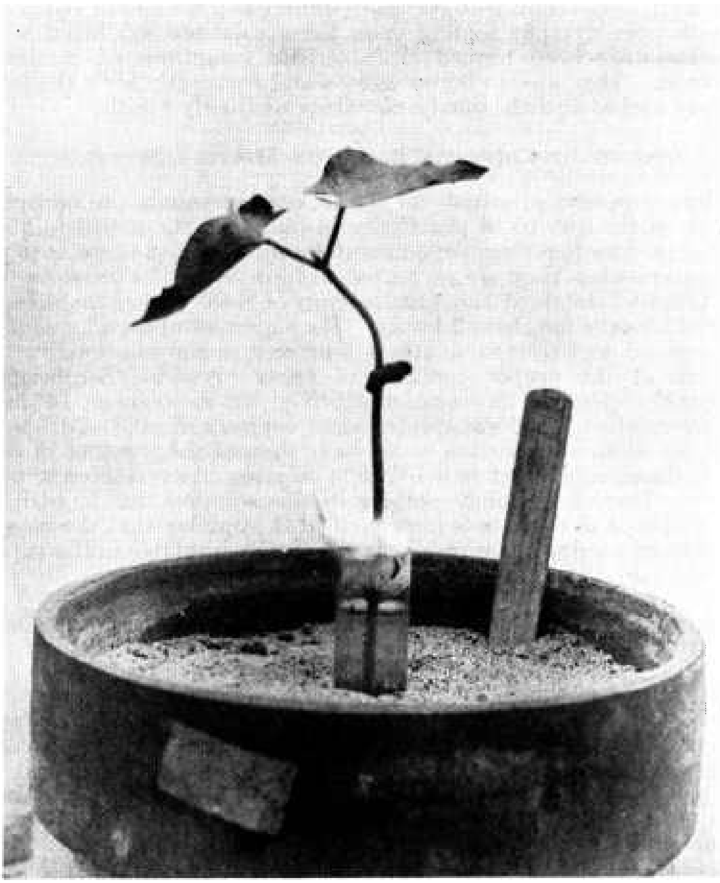

FIGURE 3.—Method used to permit leafhoppers to feed on plants whose roots were placed in chemical solutions.

in water used as untreated checks gave only 15-percent mortality over a 10-day period.

Some little variation in percentages in all toxicity tests when nymphs were used is undoubtedly due to a variation in the size of nymphs and the difference of age within an instar. Less feeding is performed just before molting, and the length of time required for death is thus prolonged in some individuals.

ANALYSIS OF LEAVES FOR COPPER

Leaves were removed at 3-day intervals from the plants whose roots had been placed in copper sulfate solutions, and tested for copper. If the nymphs died in less than 3 days on the stronger concentrations, the leaves were examined at the time of noticeable toxicity. This test was made by expressing the plant juice from the leaves and removing the proteins by precipitation. Copper was then precipitated in appreciable quantity from plants that had been held in solutions up to and including the 1–5,000 dilutions. Both the hydrogen sulfide and the potassium ferrocyanide methods were used and positive reactions were obtained.

This series of experiments has no practical bearing upon spraying with bordeaux mixture, but it has demonstrated conclusively that copper is easily taken into the plant system and as such is highly toxic to the leafhoppers feeding upon the plant, and that at the time of the insects's death the copper can be detected in the plant juices by chemical analysis and precipitation.

Effects of Copper Sulfate and Calcium Hydroxide on Leafhopper Nymphs

To test the effect of different constituents of bordeaux mixture on leafhoppers, sprays of copper sulfate at different strengths were each applied to specific plants each day for 7 consecutive days. The sprays ranged in strength from 1–500 to 1–10,000 on the different plants. After the seventh spray, leafhopper nymphs were confined to these plants. About 50 percent of the nymphs reached maturity but those that died showed typical symptoms of what has previously been termed "bordeaux death." They dropped from the plants and were unable to regain their position, or to remain there if placed on the plant. In addition, they died in a partly molted condition.

Plants sprayed thoroughly with calcium hydroxide at the rate of 6 pounds to 50 gallons of water showed no injury, and leafhopper nymphs were not affected by the lime. Furthermore, nymphs that hatched from eggs that were in the tissues of these plants when they were sprayed grew to maturity upon the same plants without being affected.

Tests With Other Copper Compounds

Three other spray materials containing copper were used on a small scale to test their effect on leafhopper nymphs and the bean plants. Different plants were treated with each of the following three materials: (1) Ammoniacal copper carbonate (copper carbonate, 6 ounces; ammonia, 3 pints; water, 50 gallons); (2) Burgundy mixture (copper sulfate, 1 pound; sodium carbonate, 1½ pounds; water, 50 gallons); (3) copper soap spray (copper sulfate, ½ pound; fish oil soap, 3 pounds; water, 50 gallons). The copper content of none of these sprays exceeded one-fourth that of bordeaux mixture prepared according to the 4–6–50 formula.

Nymphs died with typical symptoms of bordeaux killing on plants treated with each of these spray materials. The Burgundy mixture killed the highest percentage (about 66 percent), which was to be expected, since the copper content is higher in the Burgundy mixture than in either of the other two solutions.

Slight injury to the plants occurred in some cases.

Relative Toxicity of Various Strengths of Bordeaux Mixture and Their Effect Upon the Plant

Standard bordeaux mixture (4–6–50) was used in most of the field tests, but in some cases stone lime was used according to the old 4–4–50 formula. Comparative tests were made in the laboratory with different quantities of copper sulfate ranging from 1 to 5 pounds. By summarizing all the tests made with each strength of bordeaux mixture, the following results were secured:

Strength of bordeaux mixture used:	*Percentage of leafhoppers dead 6 days after treatment*
1–2–50	86
2–4–50	90
3–5–50	100
4–6–50	94
5–8–50	90

These differences in the percentage of control should not be considered as indicating any particular difference in the effectiveness of the various mixtures of different strengths. The results would indicate, however, that even a relatively small percentage of copper in the bordeaux mixture is effective for leafhopper control, probably because it acts through the plant, and that it is probably unnecessary to use as strong bordeaux mixture for leafhopper control as has been the custom in the past. Data taken from field plots of beans treated with these same strengths of bordeaux mixture agree with all the detailed experiments regarding relative toxicity. The work of Cook (*13*) showed similar results from the standpoint of stimulating effect upon the yield of the potato plant. He used various strengths of bordeaux mixture and found that the potato tubers taken from plots upon which a 2½–2½–50 spray was applied gave the highest percentage of solids, while the plots receiving a 10–10–50 spray were lower in the percentage of solids. His results indicate that a certain proportion of copper in a spray gives the maximum stimulating effect and that a greater percentage of copper may produce a toxic rather than a stimulating effect. He also obtained similar data for the number of spray applications, showing that a small number of sprays produce more solids in the tubers than twice as many applications of the same strength.

Solution of the Copper in Bordeaux Mixture and its Penetration Into the Plant Tissue

As has been indicated, the previously recorded experiments with bordeaux mixture point to the probable poisoning of leafhoppers by the juices they extract from the plant tissues. What the toxic material in the plant is and how it is established there are problems that have been difficult to approach, and they remain unsolved. It has been shown that leafhoppers die when fed on copper sulfate solutions, and on plants sprayed with copper sulfate and other copper salts, with typical symptoms of bordeaux toxicity. On the other hand, the leafhoppers that fed on calcium hydroxide and solutions of calcium nitrate and plants sprayed with calcium salts did not die in sufficient numbers to justify considering calcium as the agent responsible for the toxicity of bordeaux mixture, nor were there the symptoms typical of bordeaux poisoning. If only one of the constituents of bordeaux mixture is directly responsible for the toxicity, it apparently is the copper. It is probable, however, that no one constituent is responsible for this insecticidal phenomenon, but that two or more constituents unite to produce it. There is also the possibility that toxicity is produced by some physiological condition of the plant caused by the presence of bordeaux mixture but without its penetration into tho plant tissue. This is the idea of those who believe that an oligodynamic or chemotactic force is generated by an electrical reaction between the cell protoplasm and tho residue of bordeaux mixture on the leaves. The evidence that copper is absorbed by the plant is not entirely conclusive, but that secured from experiments where leafhoppers fed on unsprayed portions of sprayed leaves and died with symptoms of bordeaux toxicity is a strong indication that copper does penetrate plant tissue, at least in small quantities.

It has been proved that a spray of bordeaux mixture when dried is practically insoluble in water, and that if it is permitted to settle as a precipitate in a spray solution there is practically no copper in the supernatant liquid (*5*, *7*). To become effective as either a fungicide or an insecticide, however, it apparently must be rendered soluble. The question of how this is accomplished on the plant has been the subject of considerable experimental work and discussion. Previous work, especially by Crandall (*14*) has pointed to atmospheric conditions or meteoric waters as the most important agents in rendering copper soluble from the film of a bordeaux-mixture spray. Crandall, however, says (*14*, *p. 223*):

> * * * it is believed there are other agencies, that, operating alone, or in conjunction with carbon dioxide, are equally effective. The amount of carbon dioxide contained in meteoric waters is extremely minute, and, in cases where relatively large amounts of copper are found in solution, in waters, collected from sprayed trees, it is thought that the solvent action of carbon dioxide may have been supplemented by the action of other agencies. Possibly these agencies are the ammonium compounds, nitrates and nitrites, or some other constituent of the waters or the atmosphere; or the influence may rest in some agency entirely independent of the possibilities here suggested.

On the other hand, Clark (*10*) has stated that the epidermis of the leaves, although protected by a cuticle, is well known to be more or less permeable to the dissolved substances occurring in the cell sap, particularly along the union of the epidermal cells. On this basis he explains that the dew on the outside and the cell sap within cause exosmosis to take place and the copper hydroxide to become at least partly soluble.

EFFECT OF THE ATMOSPHERE AND RAIN WATER AS SOLVENTS OF BORDEAUX MIXTURE

Crandall (*14*) found that bordeaux-mixture residue exposed to the action of the atmosphere in glass dishes for 65 days failed to show soluble copper at the end of that period although the residue was moistened almost daily with cistern water. However, he did find soluble copper in rain water collected from trees sprayed with bordeaux mixture. When trees were protected from meteoric waters by a waterproof covering he was able to obtain smaller quantities of copper from bordeaux-sprayed trees by spraying or syringing the trees later with both cistern water and distilled water. This same cistern water failed to dissolve copper from bordeaux residue in glass dishes.

In the writer's experiments, in order to test the effect of meteoric waters, carbon dioxide, and other components of the atmosphere under outdoor conditions on the solubility of bordeaux mixture with the plant eliminated as a factor, heavy applications of bordeaux mixture were made to filter papers placed in glass funnels. A pint of 4–6–50 bordeaux mixture was run through these filters. The filtrate was tested for copper and gave no reaction. The stem ends of these funnels were then inserted in stoppered glass bottles and these were placed in the open where they would be exposed to the elements. From July 7 to August 5, 2.75 liters of rain water from six rains was collected in these bottles. Table 5 shows the results of tests made to determine copper in these solutions. When a test was used that would readily show any copper in a 1–50,000 solution, all results were negative in solutions as obtained or doubtfully positive even when the water was evaporated to a very small volume of concentrated solution.

TABLE 5.—*Tests for copper in rain water that had passed one filter paper coated with the residue from bordeaux mixture, 1929*

Days of exposure (number)	Rain water collected		Reaction to copper tests [1]	
	Date	Amount	Sample from total	Remainder concentrated
		Cubic centimeter		
2	July 7	710	Negative	Negative.
3	July 8	350	do	Do.
7	July 12	90	do	Indication of slight quantity.
9	July 14	70	do	Do.
24	July 29	465	do	Do.
31	Aug. 5	1,070	do	Do.

[1] Hydrogen sulfide (H_2S) and potassium ferrocyanide ($K_4Fe(CN)_6$) were used as tests.

The combined action of atmospheric factors and meteoric waters gave practically negligible results in these experiments.

SOLUBILITY OF COPPER FROM BORDEAUX MIXTURE IN RAIN WATER FROM SPRAYED LEAVES

Rain water was collected from plants that had been previously sprayed with bordeaux mixture. Soluble copper was detected very readily, and a strong positive reaction was obtained from the mixture when tested with potassium ferrocyanide. This same reaction was obtained when the sprayed leaves had been washed in distilled or tap water. The reaction in the latter case has been obtained within 2 or 3 days after the plants were treated. This as well as previous experiments has demonstrated that copper can be dissolved readily from bordeaux residue on plants but cannot be dissolved from residues of similar material on other objects or materials. The plant must therefore be recognized as an important factor in the solubility of copper from bordeaux mixture, and it is very doubtful whether soluble copper in any considerable quantity is released unless the bordeaux residue is in contact with the plant. Rain alone is apparently not important if we may judge from experiments and observations. In addition to the copper-solubility data already given, these experiments have demonstrated that leafhoppers will die in a short time on plants that have been sprayed with bordeaux mixture and protected from rain and dew. Furthermore, recent tests have given positive copper reactions in plant juices that were expressed from plants sprayed with bordeaux mixture where the residue had been removed by repeated applications of a 2- to 5-percent acetic acid solution until these solutions failed to give copper reactions, before the juice was expressed. These plants also were protected from rain and dew, and some factor other than precipitation must have rendered the copper soluble. Plants under exactly the same conditions but not sprayed with bordeaux mixture gave negative copper tests by the same method, even after having been washed in a solution of copper acetate. It has been observed (*15*) that death of the feeding leafhoppers occurs much sooner on the sprayed than on the unsprayed surface of the same leaf. Although no method has been devised for obtaining data, it is quite probable that the bordeaux-mixture residue becomes more soluble at the point where the mouth parts puncture the leaf for feeding. The plant juice is undoubtedly in closer proximity at these points with the residue of bordeaux mixture, at least in small quantity, and this plant juice has been proved to dissolve copper readily from such residue when in contact with it.

SUGARS AND ORGANIC SUBSTANCES AS SOLVENTS OF COPPER FROM BORDEAUX MIXTURE

It is well known that sugars and other organic substances will dissolve copper in small quantities from bordeaux mixture. Sugar in very minute quantity can be used to preserve bordeaux mixture from precipitation for several hours, but when a larger quantity is added the copper is dissolved from the bordeaux precipitate. With this fact as a basis, a series of experiments was performed in which use was made of Petri dishes covered with capping membranes and sealed over the open end (fig. 4). Equal volumes of distilled water, tap water, sap expressed from bean plants, and sap expressed from potato plants, as well as solutions of dextrose, sucrose, and other sugars ranging from 1 to 15 percent in strength, were placed in individual Petri dishes before the membranes were sealed over the top. The membranes were then sprayed with 4–6–50 bordeaux mixture and permitted to dry. The Petri dishes were thereupon inverted so that the solution inside was kept in contact with the membranes covered with the bordeaux mixture. In 2 or 3 days the membranes were removed and the liquid tested for copper. The distilled and tap water gave negative copper reactions in all tests, but in every case where sugar was present in the water positive tests were obtained, and the percentage of copper varied directly with the percentage of sugar in the solution. By colorimetric standards the percentages of copper present in certain of these solutions were calculated to be approximately these:

Liquid:	Reaction to copper test
Distilled water	Negative.
Tap water	Do.
Sucrose (2-percent)	Trace only.
Sucrose (4-percent)	Do.
Sucrose (7.5-percent)	1–50,000 (approximate).
Sucrose (15-percent)	1–3,000 (approximate).
Dextrose (2-percent)	Trace only.
Dextrose (4-percent)	1–1,000 (approximate.)
Dextrose (7.5-percent)	1–500 (approximate).
Dextrose (15-percent)	1–500 (approximate).

As will be noted, solutions of dextrose seem to have greater solvent powers on the copper in bordeaux mixture than equal concentrations of sucrose solutions. The plant juices of both bean and potato gave strong positive tests, showing that the juices of these plants can readily

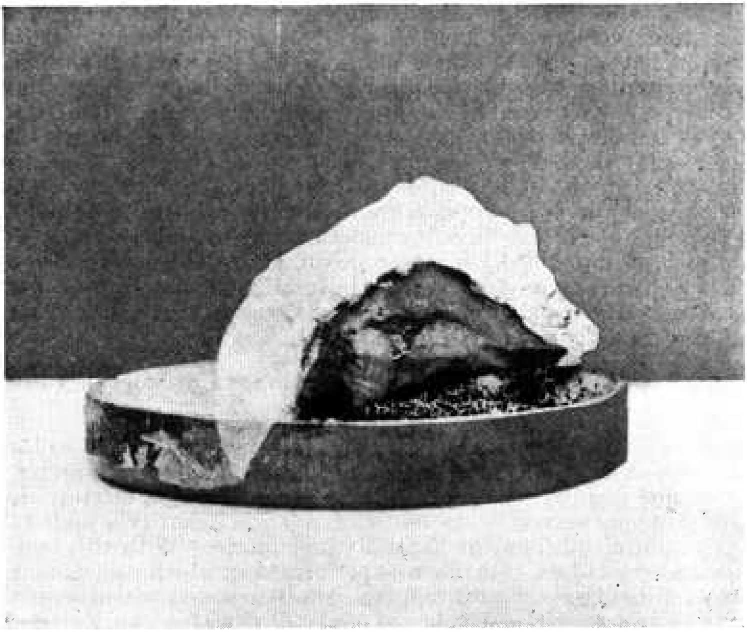

FIGURE 4.—Petri dish and partly opened capping membrane used in solubility tests

dissolve copper in some form from bordeaux-mixture residue and absorb it through a permeable membrane. A similar reaction caused either by sugars or organic acids probably occurs in sprayed plants.

REFRACTOMETER READINGS OF PLANT SAP FROM SPRAYED AND UNSPRAYED PLANTS

Tests were also made to determine, if possible, the effect of bordeaux-mixture spray upon the sap of treated plants. To accomplish this, refractometer readings were made daily on sap from both treated and untreated plants. The leaves of both were washed in the same solutions to remove spray residue when present, then after being drained thoroughly they were pressed in a hydraulic press, a uniform pressure being applied. The plant-sap samples were then tested by the Abbe refractometer for percentages of solids (table 6). These solids are usually counted as all sugars, since the percentages of the other combined solids are extremely small.

TABLE 6.—*Computation of solids (sugars) in sap from potato and bean plants, sprayed with bordeaux mixture and untreated, from Abbe refractometer readings of plant sap,*[1] *August 1929*

Days after treatment (number)	Solids present in— Untreated potato	Treated potato	Untreated bean	Treated bean	Days after treatment (number)	Solids present in— Untreated potato	Treated potato	Untreated bean	Treated bean
	Percent	*Percent*	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*
1	5.06	4.76	6.06	6.26	11	4.18	5.48	7.08	7.48
2	4.44	3.94	3.84	4.34	12	4.68	6.18	9.58	8.58
3	3.6	3.9	6.0	5.2	14	4.54	5.04	6.84	7.54
4	4.5	4.8	5.1	6.9	15	4.78	5.48	8.48	8.28
5	3.7	4.4	4.9	5.4	16	5.08	5.18	7.98	8.18
7	3.36	3.66	5.26	6.06	17			6.34	6.74
8	3.9	4.4	6.5	5.7	18	3.3	3.3	6.9	7.0
9	3.58	4.48	5.58	6.28	19			6.9	6.8
10	4.44	4.44	6.34	6.34					

[1] Computed from 6,400 readings.

When daily examinations were made, these readings usually showed that in both bean and potato plants there was a difference in the refractive index of treated and untreated plants when they were of exactly the same age and were taken from adjacent rows where soil and atmos-

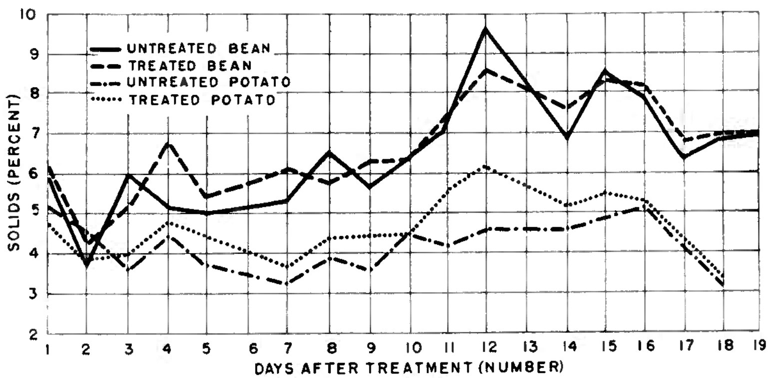

FIGURE 5.—Variations in sugar content of potato and bean plants treated with bordeaux mixture and untreated for 19 days following applications of spray.

pheric conditions were the same. Numerous examinations over periods of several days after treatment indicate that as a rule the sugar content of the treated plants is lowered below that of the untreated plants for 1 or 2 days after treatment. The sugar content, however, soon rises in the treated plant above that of the untreated and usually remains above for a period of 2 or 3 weeks, occasionally dropping below for a short period. These conclusions regarding the changes in plant sap or bordeaux-treated plants were based on some 26,000 refractometer readings made during June, July, August, and September, 1929, which represent a large portion of the growing season. A portion of these data are shown in graphic form in figure 5, which shows the results of 6,400 readings made in August. Both the age of the plant and the rate of transpiration may affect this sugar content to some extent and may change the relative percentage between treated and untreated plants. Duggar and Cooley (*20*) have shown that in potted plants the transpiration rate is higher upon treated than upon untreated plants. On the other hand, the recent work of Blaydes,[5] who worked on these same plants which were tested for sugars, has shown that bean plants in the field sprayed with bordeaux mixture

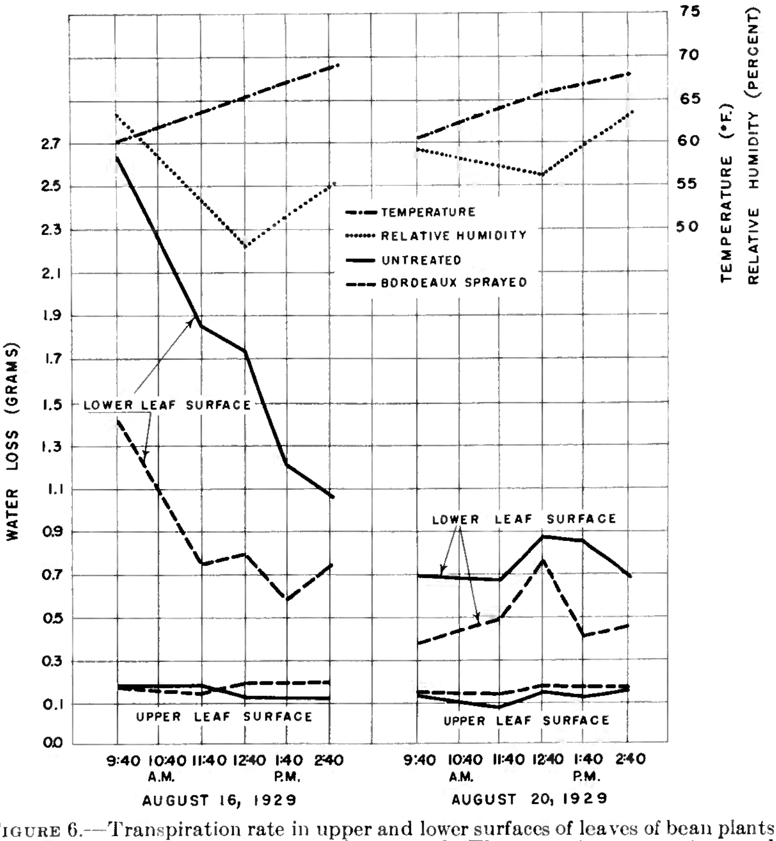

FIGURE 6.—Transpiration rate in upper and lower surfaces of leaves of bean plants sprayed with bordeaux mixture and untreated. The respective temperatures and humidities are shown by hours during the days these data were obtained. (Blaydes.)

lose much less water during the day than untreated plants in the same plots. He has shown further that the marked difference in loss occurs upon the under surface, while the upper surface shows very little loss in the case of either treated or untreated plants (fig. 6).

In very young or extremely old plants there is more fluctuation in the relative percentages of sugars, and the above-mentioned condition will frequently change from day to day.

The present data obtained from refractometer tests are in agreement with the work of Cook (*13*), who found that in potato plants

[5] Unpublished manuscript.

sprayed with copper the percentage of weight of the tubers compared to that of the vine is higher on treated than on untreated plants, the tubers being the natural outlet for the starch manufactured by the leaves. This stimulation of tuber growth and the relative increase in the weight of tubers are evident early in the development of the plant.

OTHER CHEMICAL ANALYSES

An analysis of bean leaves sprayed with bordeaux mixture and those unsprayed as recently made by E. C. Wagner in the biological chemical laboratories at Ohio State University is shown in table 7.

TABLE 7.—*Analyses of expressed sap of bean leaves,[1] untreated and sprayed with bordeaux mixture, as determined by E. C. Wagner of Ohio State University*

Constituents determined	Per 5 liters of extract		Per total dry weight		Per total green weight	
	Untreated	Sprayed	Untreated	Sprayed	Untreated	Sprayed
	Grams	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Total reserve polysaccharides as glucose			6.79	6.60	1.18	1.25
Starch			1.50	1.40	.25	.26
Total sugars	4.10	4.93	.98	.89	.82	.99
Sucrose	2.11	3.90	.50	.70	.42	.78
Reducing sugars	1.99	1.03	.48	.19	.39	.21
Insoluble nitrogen			4.30	3.50	.89	.66
Amino nitrogens	.104	.076	.02	.01	.021	.015
Amide nitrogens	.04	.036	.001	.0008	.008	.0072
Ammonia nitrogens	.026	.0095	.006	.002	.005	.002
Nitrates	.068	.0095	.02	.002	.014	.002
Total nitrogen including NO_3	.31	.26	.07	.01	.062	.052
Dry material in 5 liters of extract			20.51	17.05		

[1] There was a total dry weight of 85.76 gm. in 500 gm. of the green leaves of the untreated plants and 91.95 gm. in 500 gm. of green leaves from sprayed plants.

These data show the following significant differences in composition: (1) The bordeaux-sprayed leaves contained higher total sugars, with more sucrose but less reducing sugars than the unsprayed check plants; (2) the various forms of nitrogen were present in greater quantities in the unsprayed leaves in all cases; (3) the sprayed leaves showed a higher dry weight; (4) in general the sprayed leaves showed less nitrogen and more carbohydrate than the unsprayed leaves.

PREVIOUS EVIDENCE INDICATING ABSORPTION OF COPPER

There is little doubt that the plant is definitely affected by spraying with bordeaux mixture. Investigators, however, differ in their opinions concerning the degree to which this occurs and the way in which it is manifested. The great majority of data on yields accumulated by scientific workers over a period of 25 or 30 years indicate that the potato plant is stimulated in some way and that there is greater production on treated than on untreated plots, even when fungus and insect enemies seem to be present in such small numbers as to cause no appreciable reduction in yield on the untreated plants.

POTATO YIELDS AS INDICATORS OF CHANGES IN PLANT PHYSIOLOGY DUE TO BORDEAUX MIXTURE

The yield of a plant is always an indicator of conditions affecting plant growth and plant physiologic processes or changes. The effect of bordeaux mixture on changing potato yields should therefore furnish added proof of the physiologic changes brought about by the spray and augment the data already given in substantiating the theory of bordeaux toxicity.

Although several authors (*3, 11, 21, 27, 29, 32, 34, 35, 50, 51*) have demonstrated by field-plot experiments that bordeaux mixture increases potato yields, the most striking, detailed, and conclusive evidence has been produced by Cook (*12, 13*), who made a quantitative chemical study of the effect of bordeaux sprays on the plant in the absence of disease and insect infestations. He demonstrated that all plants contain small quantities of copper and that the tubers contain only traces of copper, whereas the roots, stems, and leaves of potato plants contain appreciable quantities. Although these individual plants show certain variation in the amount of copper present, the majority of samples from copper-sprayed plants show appreciably higher quantities than the unsprayed. This agrees with the work of Lehmann (*31*), who found that soil with a high copper content would increase copper content of the plant. MacDougal (*36*) also found that trees grown in soil with a high copper content showed metallic copper in relatively large quantities throughout the tissues.

Cook (*13*) showed, furthermore, that yields were increased by the use of bordeaux sprays, and that as a rule potato tubers from bordeaux-sprayed plots are higher in the percentages of solids, starch, and nitrogen than the tubers from unsprayed plots. These conclusions were drawn from data secured in seven States. The total starch content increased approximately 50 percent, the total nitrogen increased, and the soluble, coagulable, monoamino and amid nitrogen increased as the tubers matured.

In regard to his experimental work, Cook has this to say (*13, p. 23*):

> The results reported in this paper appear to establish the fact that copper sprays not only increase the yield of potatoes in various sections of the country, but favorably influence their composition. * * * In addition to plant-disease control and insect control, the copper appears to exert a stimulating action on the potato plant.

The data that he has given to support this work is the most conclusive offered so far by any worker.

A few workers, on the other hand, claim that the crop is reduced in yield by bordeaux sprays and they support their belief by data secured entirely by yields on field plots. Although Folsom and Bonde (*27*) previously believed that bordeaux sprays alone increased potato yields, more recent personal correspondence indicates that they now believe increased yields are due entirely to the control of plant diseases and insect pests.

In some cases it is a well known fact that conspicuous injury and destruction of foliage occur. It is apparent from the work performed by many investigators that varied conditions such as different methods of preparation of the spray, the time and number of applications, the strengths, especially if excessive, and climatic and soil fluctuations are almost certain to give varying results. The evidence seems to indicate, however, that as a rule the plant is stimulated by application of bordeaux mixture.

Although it is admitted by all workers that bordeaux sprays may increase yields through their action upon plant and insect pests of the potato, it is very difficult to explain all the increase on the basis of this protective action alone.

Various theories have been advanced to explain this increased yield, especially in potatoes. Some are as follows:

(1) One group of workers believe that the vines become more vigorous and produce greater crops because the copper sprays protect the plants and free them from fungous and insect enemies.

(2) Another group have attributed these increases to a change in the rate of transpiration. This might easily affect growth of the plant, translocation of the starches, and composition of the tubers.

(3) The rate of photosynthesis may be changed by copper sprays directly or by the shading or sunlight effects. This is claimed by some authors to explain differences in yields.

(4) A small quantity of copper may be absorbed by the plant and this might stimulate it to greater activity by changing the general rate of metabolism. There is nothing to indicate that bordeaux mixture is able to make all plants treated with it equally toxic to an insect or any particular plant toxic to several or all insects. Apple trees treated with bordeaux mixture during this study to ascertain their toxicity to *Empoasca fabae* showed no toxicity to species of *Erythroneura* or *Typhlocyba* present in abundance on the same trees.

(5) It is possible, also, that when copper penetrates into plant tissues, it may form some organic compound with some of the constituents of the cell, which, although nonpoisonous to the plant, stimulates it, protects it from fungous diseases, and immunizes it against attacks of the potato leafhopper.

It is possible, too, that copper is accompanied in its penetration of the plant tissues by some other chemical that acts as a buffer to the normal toxicity of copper toward plant tissue. R. H. True [6] has stated that the presence of calcium greatly reduces the toxicity of copper and certain other metals toward plants. Thus, if lime were absorbed with copper from bordeaux residue, the plant might be able to tolerate a greater concentration of copper than would be possible otherwise.

Cook (*13*) thinks that since copper had a favorable effect on the yield and composition of tubers and since larger quantities of copper and amino and amid nitrogen were present in copper-sprayed plants than in unsprayed plants, it is possible that "the amino acids protect the cell activity from any toxic action of the copper, thus permitting the copper to exert a stimulating effect on the cells" (*13, p. 22*).

Cook suggests this possibility in view of the work of Sherman and his coworkers (*45, 46, 47*), who have studied the effects of certain antiseptics upon amylases all of which were very sensitive to copper sulfate. They also demonstrated that certain amino acids increased the rate of hydrolysis of starch by purified amylase. By adding one of these amino acids they were able to protect the enzyme from the deleterious effect of copper sulfate and to restore to full activity any enzyme that had been partly inactivated by copper. This work may be more important in explaining the successful absorption of copper by plants without their being injured than any other data that have previously been presented.

From the foregoing data it is quite evident that bordeaux mixture affects the plant decidedly, both physiologically and chemically, and causes measurable changes to take place. Since it has been definitely proved by feeding tests that copper sulfate solutions are not toxic to

[6] In a lecture delivered before the Graduate School of Ohio State University.

insects in concentrations lower than 1–10,000 and since copper can be found only in very minute quantities (less than 1–50,000) in expressed juices from sprayed plants, it seems reasonable to believe that toxicity is due to some physiologic change and that the plant is induced by these sprays to produce a temporary immunity.

SULFUR AS A LEAFHOPPER INSECTICIDE

Sulfur in different forms has been used for several years as an insecticide, especially in the form of lime-sulfur as a control for scale insects. Several theories have been advanced to explain its action as an insecticide, the two theories that have received most attention and favor being, (1) that it is through the oxidation of the polysulfide materials together with the accompanying chemical and physical changes and (2) that it is through the production of sulfur dioxide as a toxic agent from practically all types of sulfur. So far as can be ascertained from the literature no one has considered the plant as a factor, but sulfur products, like bordeaux mixture, seem to work through the plant to produce their toxic action on the insects, and the plant becomes temporarily immunized against the attacks of these sucking insects.

Insectary Tests With Sulfur

A series of experiments were performed to test the value of certain sulfur sprays as leafhopper insecticides on bean plants. The three sprays, used in several replications, were made from dry lime-sulfur, wettable sulfur, and a spray made up with dry flotation sulfur, Bordeaux mixture was also used as a control with some of these tests.

The following four methods of treatment were used in these tests: (1) Insects were treated and placed upon untreated plants; (2) plants were sprayed with insects upon them; (3) plants were treated and insects placed upon them, either immediately after they were dry or within 1 to 26 days after they had been treated; (4) plants containing known populations were placed under bell jars and exposed to fumes from the sulfur spray materials in Petri dishes beneath the plants.

Detailed data were obtained concerning the length of time required for death of the leafhoppers and the percentage that died upon these plants as compared to the mortality on the check plants. All test insects were placed in screen cages after treatment. Since these tests were carried on continuously from August 12 to October 3 with the natural changes in temperature and humidity, it is difficult to summarize the results definitely. The averages, however, are indicative and these are given, and certain specific data are pointed out that may show the value of certain of these materials and indicate the manner in which they function.

In tests in which the insects were treated with sprays of the strength used in the field and placed upon untreated plants, lime-sulfur spray at 1 gallon to 50 gallons of water gave 60 percent mortality in 11.5 days, flotation sulfur spray at 5 pounds to 50 gallons of water gave 83 percent mortality in 15 days, and wettable sulfur at 2½ pounds to 50 gallons of water gave 65 percent mortality in 14 days. The check showed 45 percent mortality in 10.5 days. From these tests it is apparent that only slight mortality is produced by applying these materials to the insect.

In view of the fact that the detailed tests showed practically no difference in the results where the insects were present on the plants when the latter were sprayed (method 2) and where they were placed on them after treatment (method 3), the data from these tests are briefly summarized together and then specific instances will be cited.

Fourteen tests, involving some 200 nymphs, where plants were treated with lime-sulfur showed a 100-percent mortality in 5.8 days, or 81 percent mortality in all tests in 4 days. The checks for all tests gave 45 percent mortality in 10.3 days.

Fifteen tests, also with about 200 nymphs, where plants were treated with flotation sulfur spray showed 100-percent mortality in 6.6 days, or 86 percent mortality in 4 days.

Some 200 nymphs were used in 17 tests in which plants were treated with wettable sulfur. These tests showed 100 percent mortality in 8 days or 68.7 percent mortality in 4 days. These are to be compared with the same checks as the foregoing.

As specific instances of cases where lime-sulfur spray was used, leafhoppers placed on the plant 5 days after it had been sprayed showed a 70-percent mortality in 4 days and 100-percent mortality in 7 days. Insects placed on another plant 6 days after treatment showed 92-percent dead in 4 days and all dead in 5 days, and when insects were placed on another plant 13 days after treatment there was a 60-percent mortality in 4 days and 100-percent in 6 days. In another case the leafhoppers were not placed upon a plant until 26 days after it had been sprayed, but in 6 days 85 percent were dead and in 10 days all were dead.

Where flotation sulfur was used in the spray, leafhoppers were placed on the plant 5 days after it had been treated and 80 percent died in 4 days and 100 percent in 5 days. When the insects were placed on the plant 7 days after treatment 90 percent died in 4 days and 100 percent in 5 days. Another test, in which the plant was treated 11 days before the insects were put on it, gave 90-percent mortality in 4 days and 100-percent in 9 days. Where insects were placed on the plant 13 and 18 days after treatment 100 percent died in 4 days.

About the same result was obtained when plants were sprayed with wettable sulfur. Insects placed on the plants 6 days after treatment all died in 7 days, and there was a 60-percent mortality in 4 days. When placed on the plant 7 days after it had been sprayed there was a 95-percent mortality in 4 days and 100 percent died in 5 days.

Method 4 was the exposure of leafhoppers on potted plants to fumes from sulfur spray materials which were placed in Petri dishes beneath the plants and bell jars placed over the plants. The same three sulfur products were used in these tests and water was placed in the Petri dishes under the check plants. The mortality of the leafhoppers on the plants where sulfur was used was no greater than on the check plants. This indicates that the sulfur fumes alone are not responsible for the higher mortality produced by sulfur sprays.

From the tests and observations on the various phases involved, chemical, biological, and physiological, it seems apparent that changes take place in the plant through the changing of plant processes. It is the belief of the writer that the residual insecticidal effect is not due to a volatile product from the film of sulfur on the plant, but to this changed condition in the plant. This belief is substantiated by

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the facts that when plants are treated 3 or 4 weeks before the insects are placed on them, a high mortality of the insects results in a very few days, and also that these same sulfur sprays did not affect the insects when exposed under bell jars in evaporating dishes located just beneath plant leaves that were infested with leafhopper nymphs.

As a summary of these tests with the three forms of sulfur it may be stated (1) that leafhoppers treated with sulfur sprays and placed on untreated plants were only slightly affected by the sulfur; (2) that where the plants were sprayed with sulfur products with the insects upon them the mortality was very high; (3) that if the untreated insects were placed upon treated plants immediately after treatment, or from 1 to 26 days after treatment, the mortality was approximately the same, and high in every case; and (4) that when leafhoppers were placed on untreated plants and exposed to the volatile products of the sulfur sprays by placing these sprays in Petri dishes beneath the plants and covering the plant with a bell jar cage, the mortality was no greater than on the check.

FIELD TOXICITY STUDIES WITH BORDEAUX MIXTURE, PYRETHRUM, AND SULPHUR

During the summers of 1932 and 1933 field studies were made in Ohio, and during the winter of 1933 work was performed in Florida to determine, if possible, the effect on the populations of leafhoppers when various materials such as bordeaux mixture, sulfur products, and pyrethrum sprays and dusts were applied under field conditions.

Methods of Estimating Leafhopper Populations

The method used for determining the populations of leafhoppers was to count the nymphs present on 25 or 50 trifoliate bean leaves on each plot, picking these leaves or an equal proportion on the same relative level on each plot but choosing the plants in any respective plot entirely at random. The nymphs in these counts were divided into two groups. The fourth-instar and fifth-instar nymphs were designated as large, while the first-, second-, and third-instar nymphs were designated as small. Also the number just hatched was indicated, especially where the numbers on spray plots had been greatly reduced or entirely killed by spray materials. Since the leaves contain eggs that will hatch over a period of a week or 10 days after treatment, data can be obtained concerning (1) the effect of any material on the viability of eggs, and (2) the residual or carry-over effect on the young nymphs after they have emerged. For instance, if young nymphs which have just emerged are found when counts are being made on succeeding days, but no larger nymphs are present, it is indicative of a residual toxicity. This is verified by the finding of the young nymphs dead upon the leaves. The graphs are then constructed on the basis of the number of live nymphs per 100 trifoliate leaves.

In view of the fact that the adults shift readily from plant to plant and the nymphs shift very little, the counting of the nymphs and disregarding of adults is considered to give the best index to relative populations. Furthermore, if the adults are present in different numbers, the egg deposition and subsequent hatching during the period under study will usually give a better indication of this than an attempt to count the shifting and active adults.

In the laying out of plots, soil or moisture conditions, if variable, differences in plant growth conditions, and variability in populations were considered. By making population counts throughout the field and choosing plants at random in the various areas, the original populations could be fairly well determined on all plots, and a sufficient number of check plots were then chosen to represent every different condition.

Treatments were all made by applying sprays or dusts to the under side of the leaves, and so far as possible uniform conditions were chosen for all plots of a given series.

Field-Control Experiments in Ohio in 1932

A series of experiments was planned in Ohio in 1932 to test the toxicity of bordeaux mixture, sulfur, and pyrethrum sprays to the

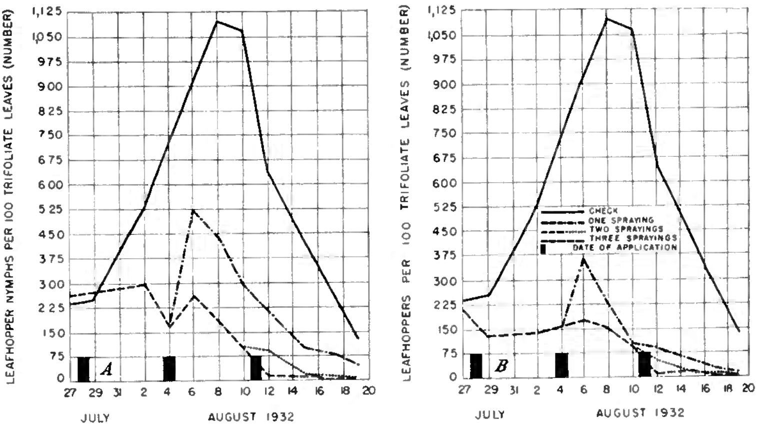


FIGURE 7.—Effect of spraying on the numbers of *Empoasca fabae* on bean plants, Ohio, 1932: *A*, with 4–6–50 bordeaux mixture; *B*, with 3–4½–50 bordeaux mixture.

potato leafhopper, and to observe the consequent reduction in the leafhopper populations.

Each plot was divided into three portions, A, B, and C. Each of these three received the first spray, only B and C received the second treatment, and C alone was treated with the third application. In this way the effectiveness of one, two, and three sprays, respectively could be determined in relationship to populations. The first spray was applied July 28, the second August 4, and the third August 11.

In all the graphs curves are included showing the effect on the populations of omitting the second and third sprays.

One plot (fig. 7, *A*) sprayed with bordeaux mixture 4–6–50 showed a slight increase in population from 268 per 100 trifoliate leaves on July 29 to 296 on August 2. During this period the numbers in the check increased from 242 to 528 per 100 trifoliate leaves. The treated population then decreased, while the check population increased. After the second application the population increased slightly, then decreased, and the third spray applied on August 11 held the population to practically zero during the remainder of the period under observation. During this time the population in the check increased to 1,090 on August 8 and dropped to 148 on August 19.

On a second plot (fig. 7, *B*), sprayed with bordeaux mixture 3–4½–50, the population was decreased considerably by the first spray so that on July 29 the treated plot showed 136 leafhoppers and the corresponding check plot 242. The population of the treated plot continued to remain at about this level until August 4, when it was 152 as compared with a check population on the same date of 742. After the second spray there was a slight increase, and then a gradual decrease to practically zero on August 12, while the check continued to increase to 1,090 on August 8 and then decrease to 640 on August 12

A third plot (fig. 8, *A*) was treated with bordeaux mixture 2–3–50. The first application decreased the population to 84 on July 29 as compared to 148 on the corresponding check plot. The second

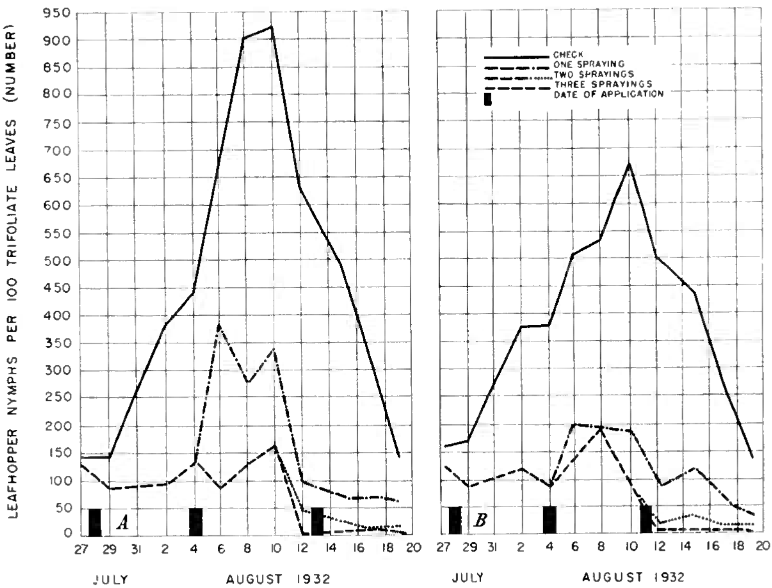


FIGURE 8.—Effect of spraying on the numbers of *Empoasca fabae* on bean plants, Ohio, 1932: *A*, with 2–3–50 bordeaux mixture; *B*, with 1–1½–50 bordeaux mixture.

application on August 4 again reduced the population to 84 on August 6, from which point it again increased to 156 on August 10. The numbers in the check at this time had increased to 916. The third spray reduced the population to zero, near which it remained until observations were terminated on August 19.

A fourth plot (fig. 8, *B*) was sprayed with bordeaux mixture 1–1½–50. The first spray reduced the population somewhat so that on July 29 the sprayed-plot population was 92, whereas the check showed a population of 172. The population of the sprayed plot remained practically the same until August 4, when it was 84, but during this time that on the check had increased to 376. After the application of the second spray on August 4 the population increased to 188 on August 8 and then dropped again to 80 on August 10, while the numbers in the check were continuing to increase to 669. After the third spray on August 11 the population on the treated plot continued to decrease to 4 on August 12 and remained at this low figure until the experiment was terminated.

The fifth plot (fig. 9, *A*) was treated with a commercial alcoholic extract of pyrethrum and coconut-oil soap diluted in the proportion of 1 to 600. The first treatment reduced the population very markedly. On July 29 that on the treated plot was 8 per 100 trifoliate leaves as compared with 176 on the check plot. From then on to August 2 the increase in the population was at about the same rate as that on the check plot and the two curves as shown in the graph are almost parallel. On August 4 the treated population had increased to 360 while the check, which had increased to 470 on August 2, dropped to 392. The second spray reduced the population abruptly again. So on August 6 the population was 16 as compared to a check

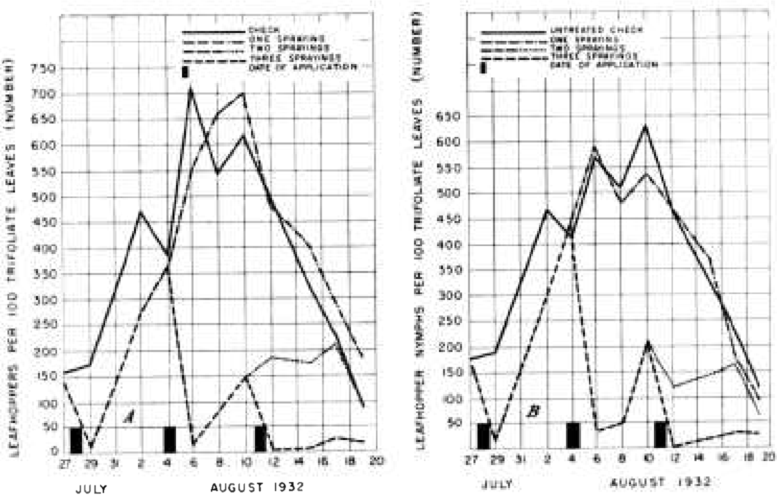


FIGURE 9.—Effect of spraying on the numbers of *Empoasca fabae* on bean plants, Ohio, 1932: *A*, with a commercial extract of pyrethrum and coconut-oil soap, 1–600 parts water; *B*, with an extract of pyrethrum 1–600, plus 1 percent of rotenone.

population of 716 on the same date. From this low population the treated plot increased to 148 in 4 days, and the third spray on August 11 reduced the population to zero while the check population was still high at 496.

The sixth plot (fig. 9, *B*) was treated with an alcoholic extract of pyrethrum at the dilution of 1–600 plus 1 percent of rotenone. The population curve shows a control curve almost exactly like that of plot 5. The first spray brought about a reduction to 4 on July 29 as compared to a check population of 188. The population rapidly increased from this low figure to 448 on August 4, while the check infestation was 408, slightly lower than that of the treated plot. The second spray reduced the population abruptly to 36 while the numbers in the check continued to increase to 572. The population in the treated plot again increased rather rapidly to 212 on August 10 and was again reduced abruptly to zero by the third spray, and it remained practically at this level until August 19. The check curve during this time continued to increase to a maximum population of 632 on August 10 and then rapidly decreased to 118 on August 19. The addition of rotenone to the pyrethrum extract did not improve control.

The seventh plot (fig. 10, *A*) was sprayed with a commercial brand of flotation sulfur. A small immediate reduction occurred so that on July 29 the population on the treated plot was 88 as compared to 176 on the corresponding check. On August 4 the insects on the treated plot had increased to 220 while the check plot showed a population of 392. The second application further reduced the sprayed

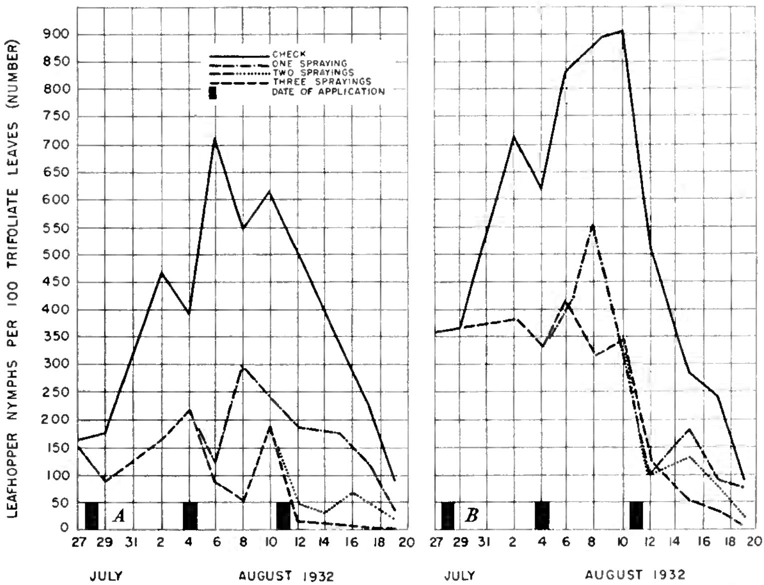


FIGURE 10.—Effect of spraying with two commercial brands of flotation sulfur, 1 pound to 50 gallons of water, on the numbers of *Empoasca fabae* on bean plants, Ohio, 1932: *A*, on plot 7; *B*, on plot 8 with a finer-ground sulfur.

population to 52 on August 8 while that on the check continued to increase to 716 and then decreased to 544. Both plots then increased. The third spray reduced the population abruptly to 16 on August 12, and it remained low until the end of the observations on August 19.

The eighth plot (fig. 10, *B*) was treated with flotation sulfur used as a spray. The first application prevented the population from increasing whereas the check increased rapidly from 368 on July 29 to 708 on August 2. After the second application the population increased slightly to 408 on August 6, then decreased to 348 on August 10. A rapid decrease to a population of 132 on August 12 followed the third application and there was a further gradual reduction to 8 on August 19. During this period the check population rose to 904 on August 10, then decreased rapidly to 96 on August 19.

The ninth plot (fig. 11, *A*) was treated with lime-sulfur. Following the first spray the treated plants showed a population of 92 on July 29 as compared to a check population of 176 for the same date. The population of the sprayed plot gradually increased to 208 on August 4, while the check showed a population of 392. A second application reduced the population to 24 on August 6, while the check population continued to increase to 716. From August 6 to 10 there was an increase on the treated plot to 108, which the third spray reduced to 12 on August 12, and it remained low during the remainder of these observations. The check showed a population of 616 on August 10 and gradually decreased to 92 on August 19.

The tenth plot (fig. 11, *B*) was sprayed with wettable sulfur. A noticeable reduction in numbers was indicated on August 2; at this

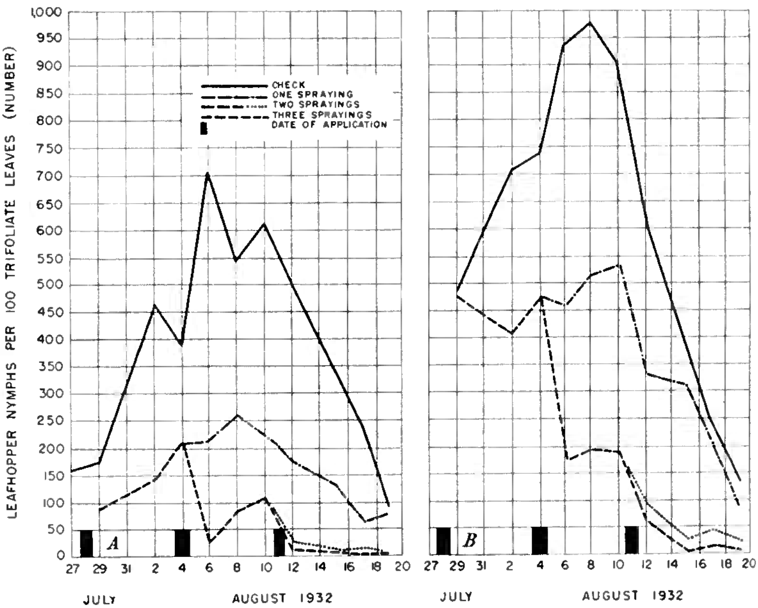


FIGURE 11.—Effect of spraying on the numbers of *Empoasca fabae* on bean plants, Ohio, 1932: *A*, With lime-sulfur, 3½–50; *B*, with wettable sulfur, 2½–50.

time the treated plot showed a reduction from 482 to 404, while the check had increased to 708, but at the time of the second application the treated plot had approximately the initial population. The second application reduced this from 476 on August 4 to 172 on August 6, while the numbers in the check rose to 934. The treated-plot population remained approximately stationary until August 10 after which the third application reduced the population still further to 60, and it continued to decline to 4 on August 15, where it remained until August 19. During this time the check population rose to 976 on August 8 and decreased to 136 on August 19.

These plot tests gave certain indications concerning the comparable value of these spray materials.

Bordeaux sprays in all dilutions used gave decided reductions in populations or prevented increases that were in any way comparable to the populations on the check plots. The check populations were variable and so were those upon the different bordeaux-sprayed plots, but all indicated the same general situation. One application reduced the population markedly, and two applications gave an economic control of the infestation in this field.

The pyrethrum sprays gave an immediate reduction in populations to practically zero, but since the eggs present in the leaves up to the time of spraying were not affected, the populations increased rapidly, and where only one application was made the peak of the population reached approximately the peak of the check. The second application again reduced the populations to near zero, but a considerable increase in the population occurred after this spray. A large amount of feeding occurred on these plots even after the application of two sprays.

In the case of the sulfur sprays there is a considerable difference varying with the type of sulfur used. Two flotation dusts were used in wet sprays on plots 7 and 8. Both gave rather marked reductions in leafhopper populations, but the newer and finer flotation dust used on plot 8 gave a little better result. The lime-sulfur spray gave a decided reduction in the population but showed some foliage injury during part of the treatments. Two applications gave a very good reduction and practical control. The wettable sulfur gave equally good or better results than the flotation sulfur. Two applications gave a very marked reduction in the population and a good economic control.

The value of bordeaux-mixture sprays and sulfur sprays as compared with pyrethrum sprays is that the former have a residual or carry-over effect that not only kills the nymphs and adults present but continues to function as an insecticide after the eggs that are embedded in the leaves hatch. This apparently is due in both cases to some condition correlated with the physiology of the plant. Experimental data have shown that it is apparently not due to a volatile product in either case, nor is it necessary that the insects be hit with this material. If the plants are treated and the insects placed on them several days later they are found to die in approximately the same time as if they were present on the plant and were hit by these sprays.

Field Tests in Florida in 1933

During February and March 1933 several series of field tests were performed on the eastern coast of Florida near Lake Worth. This is a concentrated truck-crop district, and beans are grown in rather large acreages. The purpose of these experiments was to determine whether bordeaux mixture was effective as an insecticide on beans for the control of the potato leafhopper in Florida and to test further the relative efficiency and toxicity of materials that had shown the most promising results under Ohio conditions. The very large populations that could be found in Florida were thought to be important for critical tests of these materials. Both dusts and sprays were used in making the tests.

In one series of plots bordeaux mixture, pyrethrum, and sulfur sprays were used on plots of heavily infested beans on sandy soil.

Six spray materials or dilutions were used in the treatment of these plots. These were bordeaux mixture 3–4½–50, bordeaux mixture 4–6–50, pyrethrum 1–600, flotation sulfur 5 pounds to 50 gallons of water, lime-sulfur 3.5 pounds to 50 gallons of water, and wettable sulfur 2.5 pounds to 50 gallons of water. Applications on each treated plot were made on March 2, 9, and 16. Three check plots were left untreated. Population counts of each plot were made the day before application and every 2 or 3 days thereafter until March 22, at which time the last count was made. A graph (fig. 12) showing the effect of each of the three spray treatments, together with a corresponding check, indicates the relative efficiency and toxicity of the materials on these plots.

The plot treated with bordeaux mixture 3–4½–50 showed a reduction of the leafhopper population the day after treatment from 984 to 364 per 100 trifoliate leaves, while the check population remained approximately the same. The second spray caused further reductions of the population per 100 trifoliate leaves to 268, on March 11, while the check continued to rise to 2,400. The third spray again reduced the population, this time to 276, from which number it continued to decrease to 120 on March 22, while the check had increased to 7,600 and then dropped to 7,100. In the case of the plot receiving only one spray, the population increased gradually after March 5 to a maximum of 2,900 on March 20, at which time the check had reached its highest population. The plot treated only twice showed a rise in the population after March 11 to 600 on March 17 as compared to 6,000 at this time in the check.

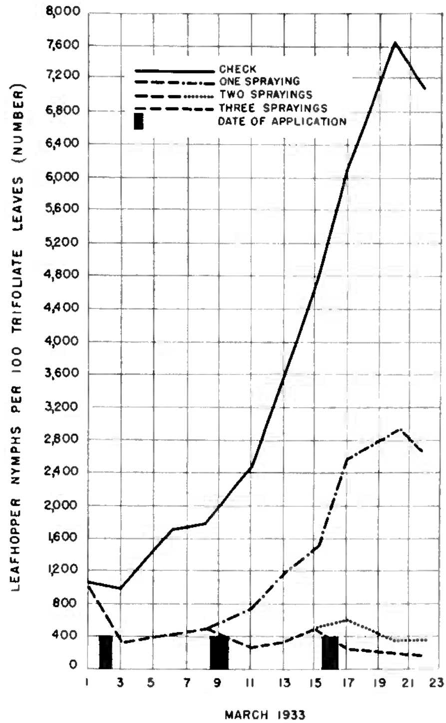


FIGURE 12.—Effect of spraying with bordeaux mixture, 3–4½–50, on the numbers of *Empoasca fabae* on bean leaves, Florida, 1933.

The plot treated with bordeaux mixture 4–6–50 gave similar results. In this case the original population of 1,044 on March 1 was reduced by the first spray to 372 on March 8, while the check population increased to 1,800. The second spray, although not reducing the population, caused it to remain at about the same level until March 13 while the check continued to increase to 3,700. The third spray reduced the population from 833 on March 15 to 112 on March 17 and the check increased still further to 6,000. The population on the plot sprayed only once continued to increase gradually to 2,032 on March 20, but at this time the check population had increased to 7,600. The two-spray plot on this same date had a population of 644 and the three-spray plot a population of 236.

The original population of the plot sprayed with pyrethrum spray 1–600 (fig. 13) was approximately 956 per 100 trifoliate leaves and this was reduced immediately by the first spray to zero. The population built up to 544 on March 8 and the second spray reduced this number to 52. There was a rapid increase in the population from March 11 to March 15 when a population of 2,268 was recorded, and the third spray again reduced the number to zero on March 17. This population increased to 624 by March 22. On the one-spray plot the population increased from 544 on March 8 to 6,870 on March 20. On the two-spray plot the population increased from 52 on March 11 to 3,800 on March 22.

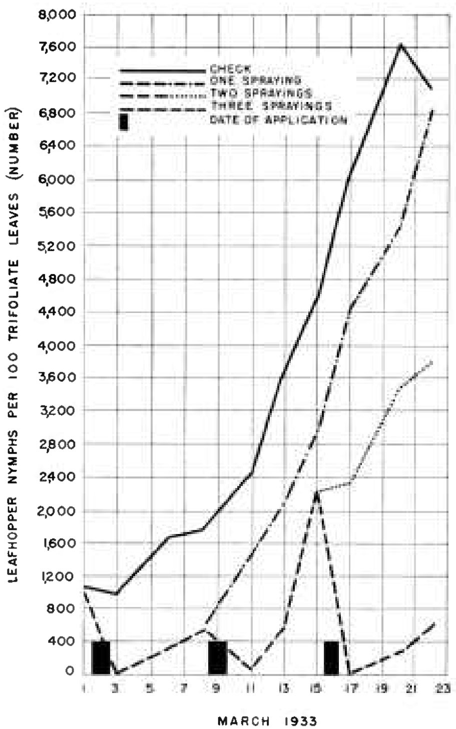


FIGURE 13.—Effect of spraying with pyrethrum, 1–600, on then umbers of *Empoasca fabae* on bean leaves, Florida, 1933.

The plot treated with flotation sulfur spray, 5 pounds to 50 gallons of water, had an original population of 628 on March 1. This was reduced by the first application to 272 on March 3. From this point the population gradually increased to 504 on March 8, while the population of the check plot had increased from an initial 894 to 1,368. In spite of a second spray the population continued to increase to 1,624 on March 15, while the check was increasing to 4,200. The third application reduced the population slightly from 1,624 on March 15 to 1,452 on March 17, from which point it continued to increase to 2,420 on March 22. During this time the check increased rapidly to a maximum population of 8,550 on March 22. On the plot receiving one application the population increased from 504 on March 8 to a maximum population of 4,960 on March 18. On the plot receiving two applications the population decreased by March 17 to 1,204 and then increased to a maximum of 2,472 on March 22.

The plot receiving the lime-sulfur spray in the proportion of 3.5 pounds to 50 gallons of water had an original population of 588 on March 1, which was reduced by the first application to 340 on March 8. During this time the check-plot population increased from 894 to 1,368. A second application did not delay very effectively a rapid increase to 1,944 on March 15, while the check population continued to increase to 4,258. A third application reduced the population decidedly to 508 on March 17, while the check increased to 5,802. From 508 the population on the three-spray plot gradually increased to 1,088 by March 22, while the check population had increased to 8,550 on March 22. The population on the one-spray plot increased at about the same rate as that on the check after being reduced to 340 on March 8. On the two-spray plot the population was slightly reduced on March 17, then gradually increased to 3,230 on March 22, while the check increased to 8,550.

The plot that received wettable sulfur in the proportion of 2.5 pounds to 50 gallons of water had an original population of 1,012 on March 1. The first application reduced this number to 708 on March 3 and it gradually increased from this figure to 964 on March 8. During the same period the check increased from 894 to 1,368. The second application did not prevent an increase from 964 on March 8 to 2,284 on March 15. The third application, however, reduced the population to 1,900 on March 17, but it gradually increased again to 2,424 on March 20. During this time the check population more than doubled, increasing from 4,258 on March 15 to 8,550 on March 22. The population of the one-spray plot increased from 964 on March 8 to 8,860 on March 22, a slightly higher population than on the check, and the increase was almost parallel on the two plots. The population of the two-spray plot increased from 2,284 on March 15 to 6,110 on March 20, then dropped to 3,980 on March 22.

DISCUSSION OF THE RESULTS

From the foregoing data certain conclusions are obvious. Bordeaux mixture at the two strengths used reduced the populations enormously, and even where only one spray was used, after more than 3 weeks' time the material still showed a residual effect on the population.

Although pyrethrum actually reduced the numbers present to a greater degree at the time of application than did the bordeaux mixture sprays, it showed no residual effect, and as a consequence the populations increased very rapidly and the population curve for the sprayed plant is almost parallel with the original or normal-population curve. After the second application a similar increase occurred but the population did not increase as much as on the one-spray plot.

The flotation sulfur showed some residual effect but not so much as in the case of bordeaux-mixture sprays. The population, however, on the one-spray plot remained much lower than in the plot where pyrethrum was used. The residual effect on the two-spray and three-spray plots resulted in decided reductions. The material had been manufactured for use as a dust but was used in spray form. Had it been applied as a dust it would undoubtedly have shown greater toxicity, judging from later applications of an ordinary dusting sulfur.

Dry lime-sulfur used in the spray form caused plant injury and failed to give an effective kill. The three-spray plot showed good control, however.

Wettable sulfur failed to give an appreciable control except on the three-spray plot. This material apparently was too easily wettable and was soon carried off the plant by heavy dews, high humidity, and dashing rains, whereas the flotation sulfur, which is very difficult to wet even slightly, gave good results.

SINGLE-PLANT REPLICATIONS WITH BORDEAUX MIXTURE

One experiment was devised to test the effect of one application of bordeaux mixture 3–4½–50 on the population of a single bean plant. Twelve bean plants were chosen at random and the populations of each plant were counted and recorded. These ranged from 105 to 358 and averaged 225.3 per plant. Checks, which were also chosen at random and counted, averaged 149 leafhoppers per plant. Each of the 12 plants chosen was sprayed on March 3, immediately after these populations were determined. The population curves in figure 14, which depict the results of the experiment, are of the same general type. Regardless of the number present at the time of application, the population of each plant decreased in 3 days so that on March 6 the populations were small, ranging from 4 to 70 for each plant, most of them being between 20 and 40, with an average of 34.8 per plant. From this date the numbers varied somewhat but only to a slight degree. Six plants showed a small increase in population, 4 plants showed a slight decrease, and 2 remained about the same. The average population at the end of these experiments was 33.5, or approximately the same average as on March 6. The check average for 4 plants gave an increase from 149 on March 3 to 261 on March 13, when observations were terminated.

Another indication given by this experiment is the actual reduction which occurs on bordeaux-sprayed plants. The average population per plant at the beginning of the experiment was 225.3 nymphs. The average 3 days after they were sprayed was 34.8 and at the end of the experiment was 33.5 per plant. This showed that one spray reduced the population 85 percent and held it to that figure for a period of 1 week, while the check was increasing 75 percent above the original population. Consequently, if the spray is given its full credit, in addition to the reduction caused by it, it also has a residual value or effect of preventing an increase in the population to the extent of 43 percent in a week's time.

PLOT REPLICATIONS WITH BORDEAUX MIXTURE

Another experiment was performed in a series of three plots picked at random, each receiving one application of 3–4½–50 bordeaux mixture. The populations were not counted at the beginning of this experiment, but counts were made first on March 6, 3 days after treatment. At this time plot 1 showed a population of 950 while the corresponding check plot had a population of 3,710. In a week's time this count increased to 1,110 on the treated plot and to 5,090 on the check, showing a final reduction of about 78 percent. The reduction on plot 2 was about the same and on plot 3 was about 70 percent.

Two series of plots were used to determine the relative toxicity of certain sulfur, tobacco, pyrethrum, and copper combinations applied as dusts. In the first series three plots were each treated with one application of dust on March 9 and the first counts were made 2 days later.

One plot received sulfur-tobacco dust in equal proportions, the tobacco containing approximately 0.75 percent of nicotine. On March 11 the population of this plot was 1,420 and the corresponding check-plot population was 2,540 leafhopper nymphs per 100 trifoliate leaves, a reduction of 44 percent. On March 20, 9 days later, the treated plot showed a population of 4,485 and the check plot a population of 7,815, or a reduction of 43 percent.

A second plot treated with sulfur-pyrethrum 80-20 had on March 11 a population of 175 as compared to a check-plot population of 1,665. On March 20 the population on the dusted plot had increased to 4,925 while the check had increased to 9,030. The initial population reduction was thus 90 percent and the final reduction was 45 percent.

A third plot treated with sulfur alone had a population of 155 on March 11 as compared to a check population of 1,510, which was a reduction of 90 percent. On March 20 this population had increased to 2,770 and the check-plot population to 7,030, which showed a final reduction of 61 percent.

These plots showed very definitely the value of sulfur dust as a leafhopper insecticide. The sulfur-tobacco dust combination gave no better results than sulfur alone; and while the pyrethrum is of decided value for immediate reduction of populations, the sulfur alone is important as a residual material and displays effective insecticidal value for a period of several days.

In the second series four plots were each treated with two applications of dusts. The first was made on March 12 and the second on March 19.

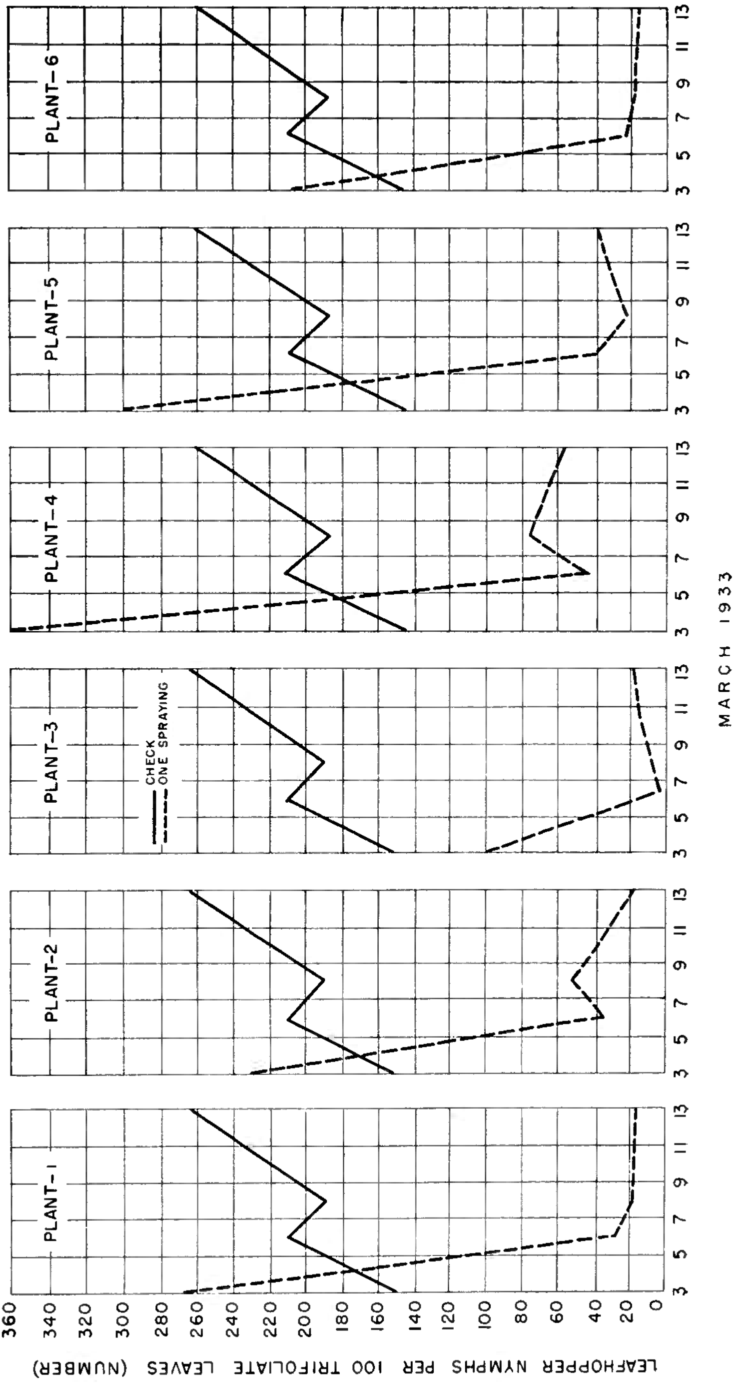
The plot receiving sulfur-tobacco dust 50–50 was examined on March 14, at which time it had a population of 2,012 nymphs per 100 trifoliate leaves as compared to a check population of 3,060, which was about a 34-percent reduction (fig. 15, *A*). The population on the dusted plot increased rapidly to 2,812 on March 16, then decreased rapidly to 808 on March 21, when the experiment was terminated. At this time the check showed a population of 6,050, which indicated that the treatment had brought about a total reduction of 87 percent on the treated plot.

A second plot treated with sulfur-pyrethrum 80–20 showed a population of 696 on March 14 as compared to a check population of 2,864, or a reduction of 76 percent (fig. 15, *B*). This population gradually increased to 1,228 on March 18 and then gradually decreased to 472 on March 21, while the check had increased to 5,585. This gave a final 92 percent reduction of the infestation.

A third plot treated with sulfur undiluted showed a population of 1,921 on March 14 as compared to a check population of 2,496, a reduction of 23 percent (fig. 16, *A*). This population gradually increased to 2,440 on March 16, then decreased rapidly and uniformly to 648 on March 21, when the check population was 5,930. The final reduction was, therefore, 89 percent.

A fourth plot treated with copper-lime dust 20–80 had a population of 1,800 on March 14 compared to a check population of 3,290 (fig. 16, *B*). This showed an initial reduction of 45 percent. On March 16 the treated population had increased to 2,488 and it then decreased to 1,368 on March 21, at which time the check had increased to 6,250. The final plot reduction was therefore 78 percent.

One of the most interesting points in regard to the graphs is the fact that the population curves for the sulfur-tobacco dust 50–50 and the undiluted sulfur are almost exactly alike. This indicates, as previous experiments had shown, that tobacco dust as well as nicotine sulfate in its usual forms are of practically no value in controlling


PLANT-1
PLANT-2
PLANT-3
PLANT-4
PLANT-5
PLANT-6
CHECK
ONE SPRAYING
MARCH 1933
LEAFHOPPER NYMPHS PER 100 TRIFOLIATE LEAVES (NUMBER)

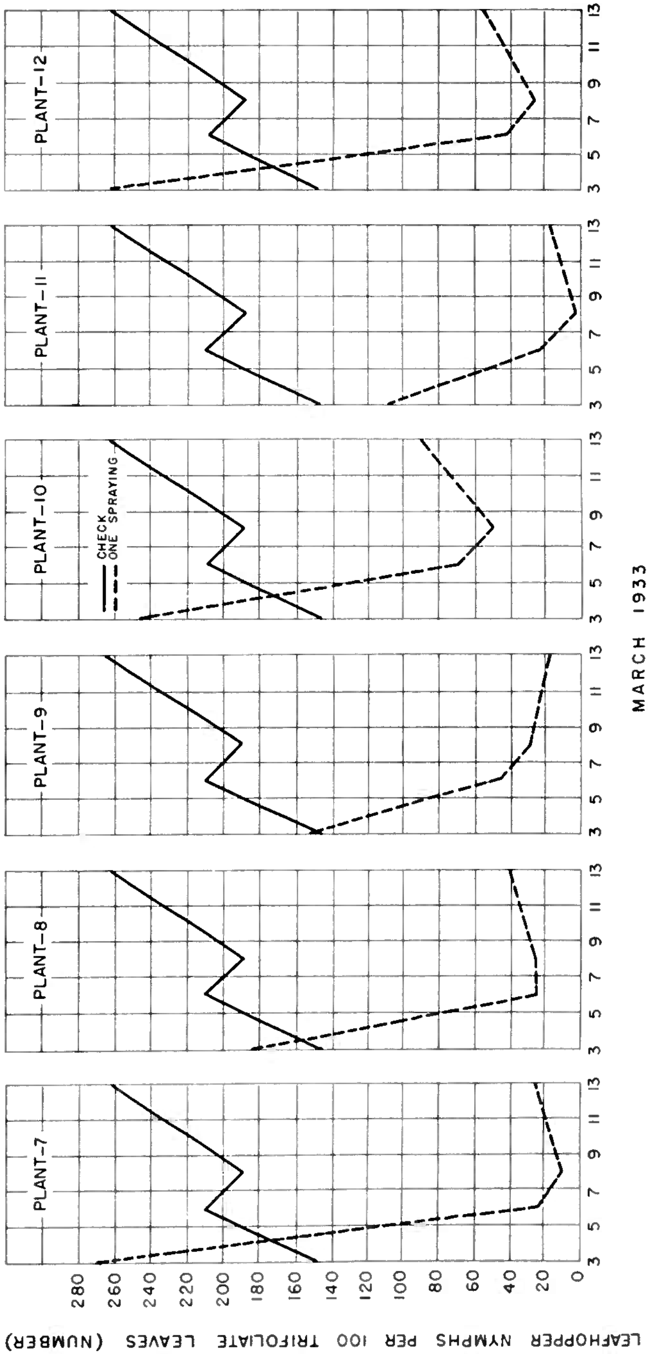


FIGURE 14.—Effect of one application of bordeaux mixture 3–4½–50 on the number of leafhoppers on 12 individual bean plants, Boynton, Fla.

Empoasca fabae. The pyrethrum-sulfur gave the best control, as would be expected, because of the decided reduction obtained immediately after treatment. The sulfur then is able to hold the population to a low percentage. Another point that should be noted is that it requires several days for the sulfur to become effective in decreasing the population. This is thought to be due to the slow effect upon the plant in whatever physiologic condition or change is established in it.

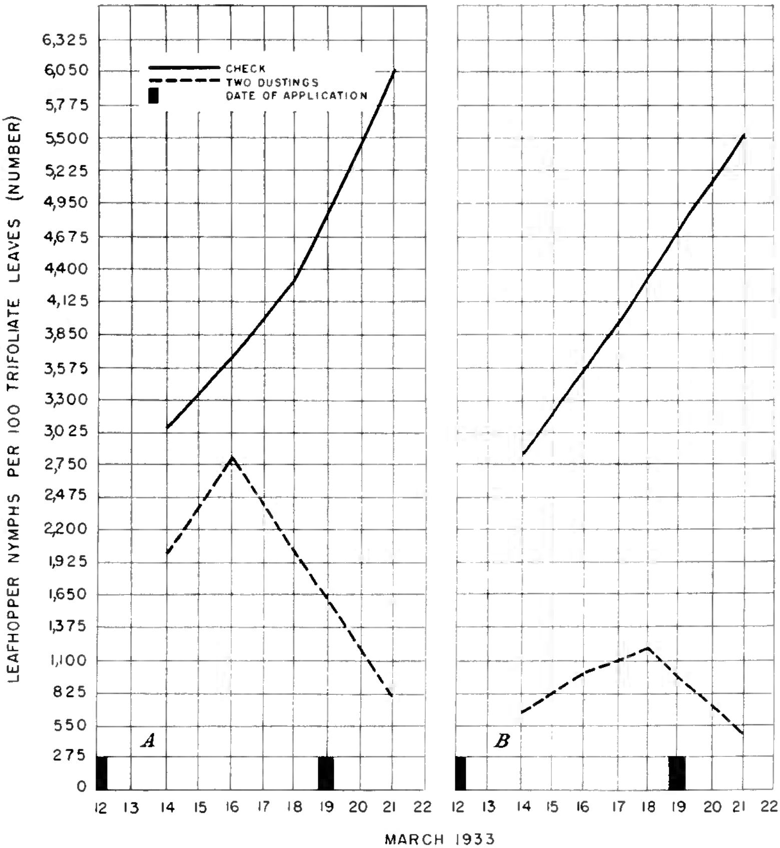


FIGURE 15.—Effect of two applications of dust on *Empoasca fabae* on bean plants in Florida: *A*, Sulfur-tobacco dust 50–50; *B*, sulfur-pyrethrum dust 80–20. March 1933.

The copper-lime-dust curve is essentially of the same type as the sulfur-dust curves and indicates that copper and sulfur affect the plant in a similar way, as indicated in the experiments carried on at Columbus, Ohio, during the summer of 1932. The final reductions of 87, 92, and 89 percent on the three sulfur plots show a slightly better control by sulfur than the 78-percent reduction on the copper plot. The pyrethrum added to the sulfur resulted in very little additional final reduction over the sulfur alone. The advantage of the pyrethrum is gained in the earlier reduction of the numbers of nymphs on the plots and the killing of many adults present at the time of application.

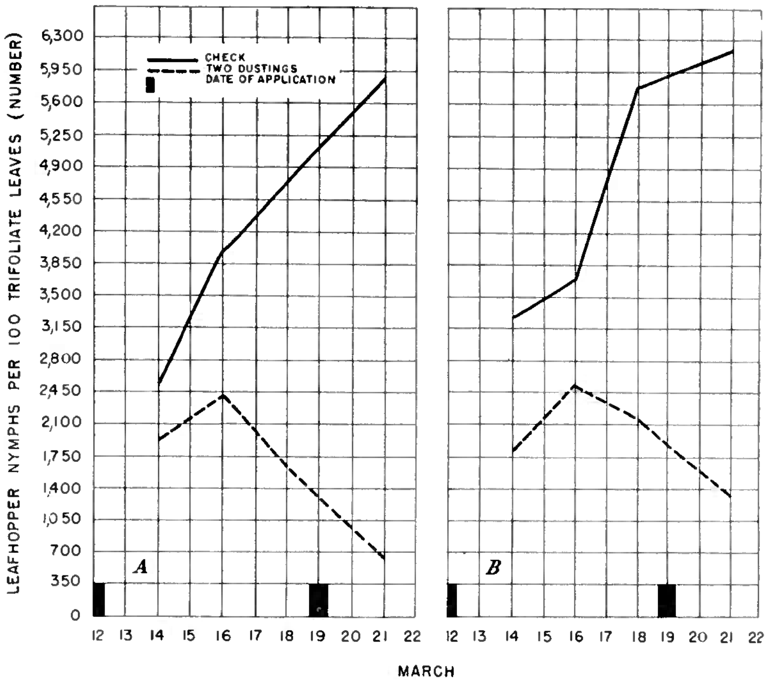


FIGURE 16.—Effect of two applications of dust on *Empoasca fabae* on bean plants in Florida: *A*, Sulfur dust; *B*, copper-lime dust 20–80. March 1933.

FIELD TESTS IN OHIO IN 1933

Further experimental studies in field control were carried on during the summer of 1933 on the farm of the Ohio State University at Columbus. Treatments were made especially with sulfur, pyrethrum dusts, and combinations of these two materials. Three different grades of pyrethrum dust were used. One contained 1.0 percent, a second type contained 0.5, and a third 0.05 percent of pyrethrins. These were used diluted in different percentages with sulfur and with each other. Since the relative values of bordeaux mixture, pyrethrum extract sprays, and flotation sulfur paste were known from previous work, a few of these were used to make comparisons where unanalyzed commercial mixtures were applied.

In view of the fact that leafhopper populations will vary considerably during different portions of the season upon beans of different ages, and where these were planted under different conditions several series of plots were used in an attempt to obtain heavy infestations for severe testing. Unfortunately weather conditions were not favorable for the production of heavy infestations during most of the season; but moderately large populations for Ohio were found during part of this time, and tests on these gave fairly reliable results or indicated certain control trends or effects. Better results were obtained during rising than during declining infestations as judged by the check populations (solid lines) on the graphs. These checks indicate the populations as found in the field naturally when untreated and undisturbed.

Since reductions of populations by spray materials have been discussed in detail in previous experiments, it seems advisable to present the results in a series of graphs, making only a general statement regarding the control trend. Bordeaux mixture 3–4½–50 (fig. 17, *A*) gave the same type of curve and population control as previ-

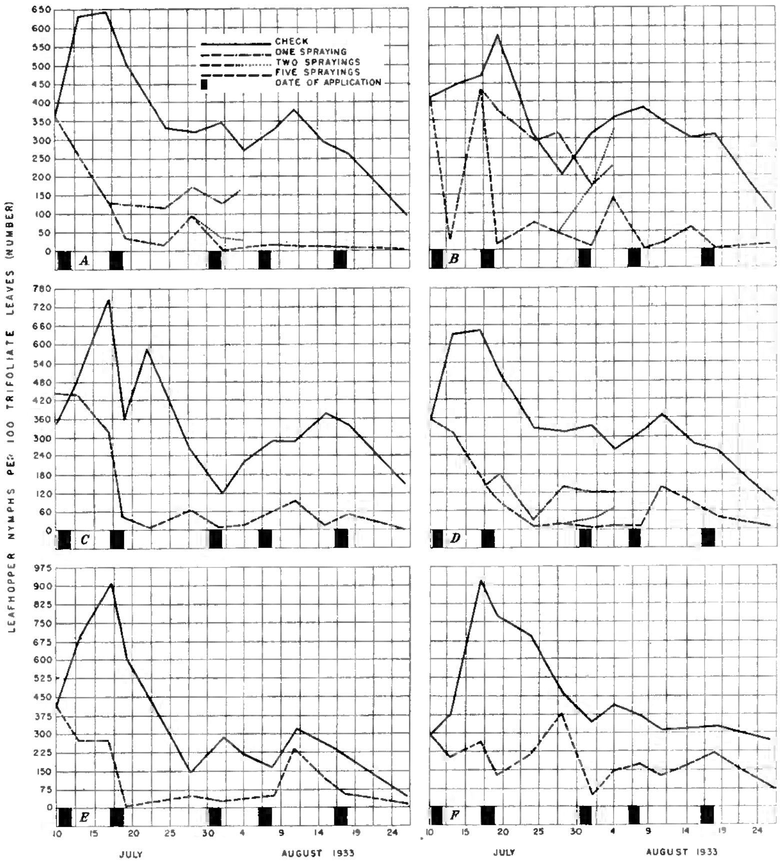


FIGURE 17.—Experiments on the control of *Empoasca fabae* on beans: *A*, With bordeaux mixture 3–4½–50; *B*, with pyrethrum extract 1–600; *C*, *D*, with a spray made from flotation sulfur paste, 8 pounds to 50 gallons of water; *E*, with a commercial brand of sulfur dust; *F*, with bordeaux mixture 3–4½–50 plus 2 percent of a white oil. Ohio, 1933.

ously indicated, reducing the population to a very low percentage and then holding it there during the entire season. In like manner the pyrethrum extract 1–600 (fig. 17, *B*) failed to give adequate control just as it had failed in previous experiments. The flotation sulfur paste 8 pounds to 50 gallons (fig. 17, *C*, *D*) gave a reduced population curve very similar to that of bordeaux mixture. A commercial brand of sulfur (fig. 17, *E*) also proved to be very good although the natural population was low in this area of the field and results were not conclusive. When bordeaux mixture 3–4½–50 was mixed with a 2-percent white oil (fig. 17, *F*) it failed to reduce the populations as much as did bordeaux mixture alone, and proved unsatisfactory.

On plots receiving only two sprays, bordeaux mixture combined with white oil (fig. 18, *D*) failed again to give as good results as

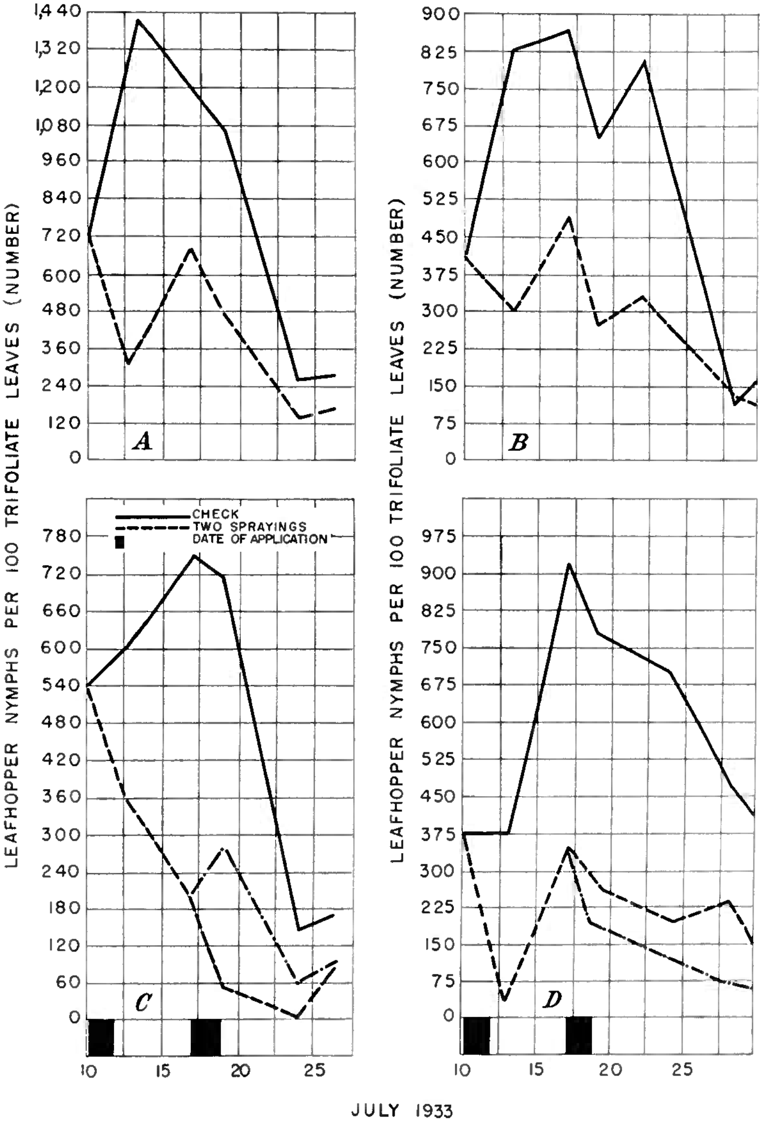


FIGURE 18.—Experiments on the control of *Empoasca fabae* on beans: *A*, *B*, With two applications of a monohydrated copper sulfate and hydrated lime, 80–20; *C*, with a commercial sulfur dust; *D*, with bordeaux mixture 3–4½–50, plus 2 percent of a white oil.

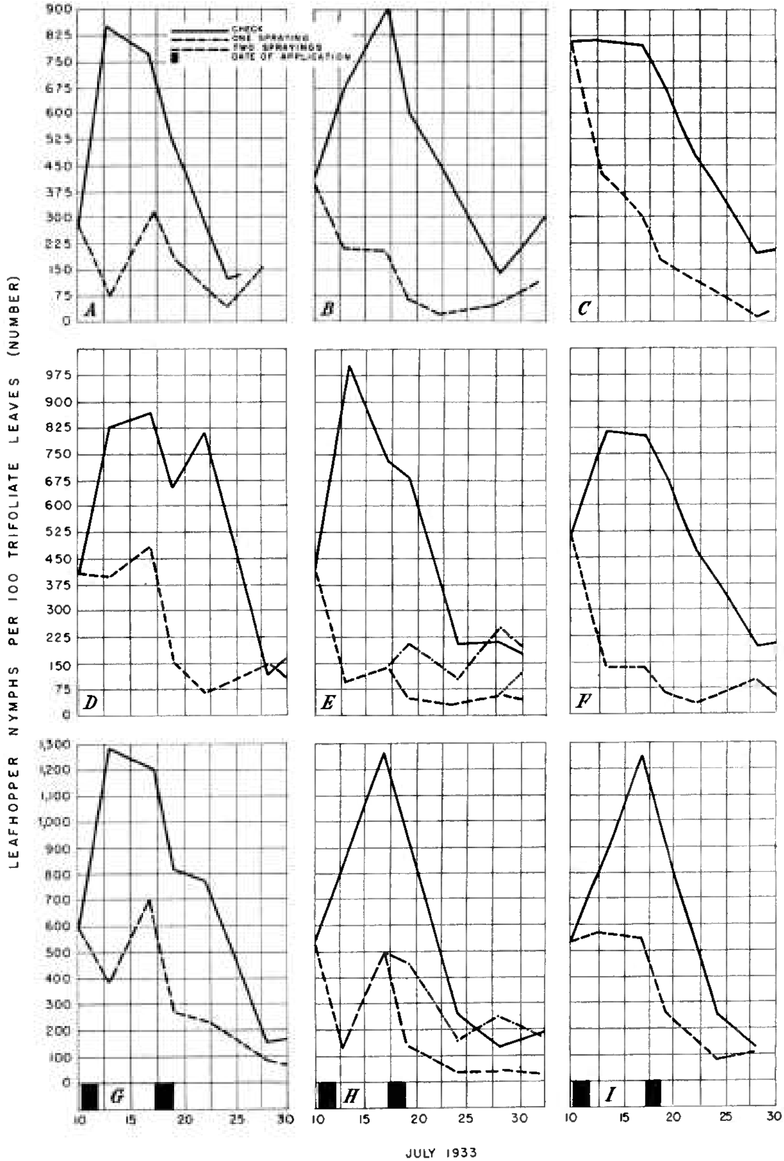


FIGURE 19.—Experiments on the control of *Empoasca fabae* on beans: *A*, *B*, Treated with two applications of flotation sulfur dust; *C* and *I*, with two applications of flotation sulfur dust and tobacco, 80–20; *D*, *E*, with flotation sulfur dust and 1-percent pyrethrum dust, 95–5; *F* and *H*, with flotation sulfur dust and 1-percent pyrethrum, 80–20; *G*, with flotation sulfur dust and 1-percent pyrethrum, 90–10.

bordeaux mixture alone. Also the commercial sulfur dust (fig. 18 *C*), although showing a decided reduction, failed to give a satisfactory control. Copper-lime dust 80–20 used on two plots (fig. 18, *A*, *B*) gave only a partial reduction in the populations as indicated by the curves on the graph.

Flotation sulfur dust alone (fig. 19, *A*, *B*) when used on two different

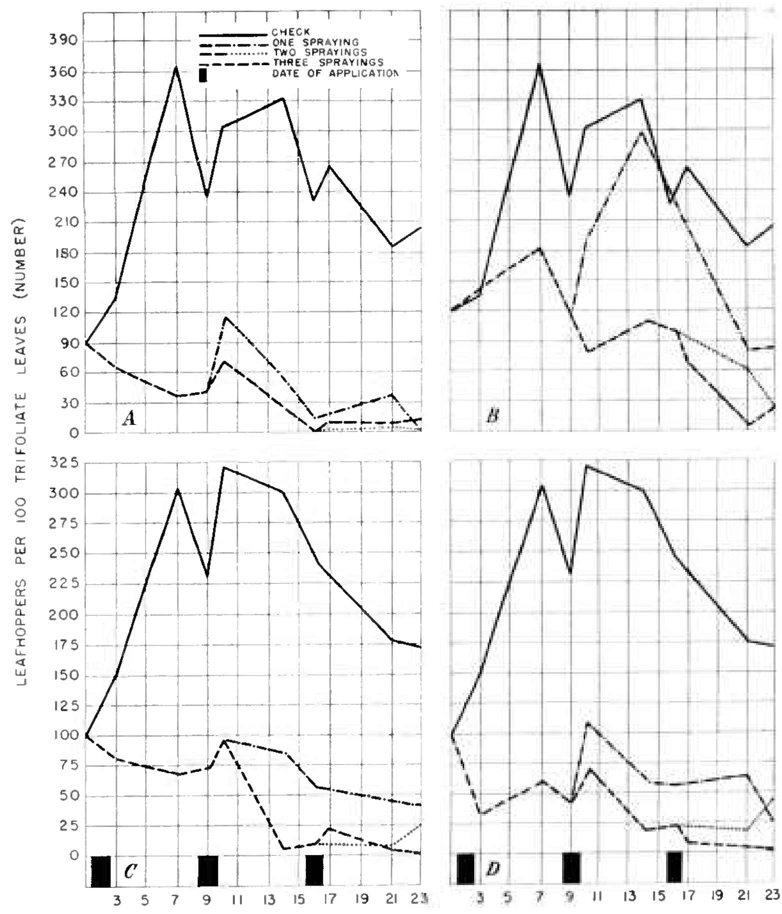


FIGURE 20.—Experiments on the control of *Empoasca fabae* on beans: *A*, With a spray made from flotation sulfur paste, 8 pounds in 50 gallons of water; *B*, with flotation sulfur dust; *C*, with dusting sulfur; *D*, with wettable sulfur spray, 5 pounds to 50 gallons of water.

plots, gave excellent reduction in leafhopper numbers but not so good as did the paste form. The flotation dust was also used in combination with pyrethrum flowers containing 1.0 percent of pyrethrins in three different proportions. One was a 95–5, (fig. 19, *D*, *E*) another 90–10 (fig. 19, *G*) and a third was an 80–20 mixture (fig. 19, *F*, *H*).

Although there was considerable variation in the population curves on the replicated plots, there seemed to be very little average difference in the results obtained from these different combinations. Flotation sulfur dust (80 parts) used with tobacco dust (20 parts) (fig. 19, *C*, *I*) failed to give any greater reduction of the populations than did flotation sulfur alone.

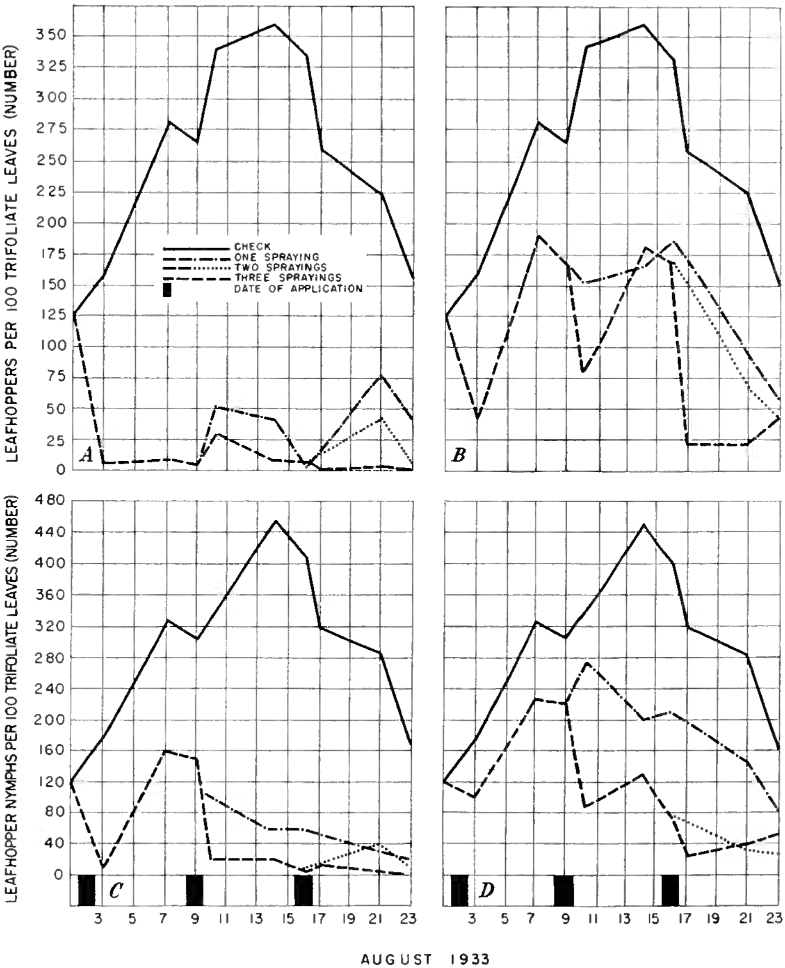


FIGURE 21.—Experiments on the control of *Empoasca fabae* on beans: *A*, With dry-mix spray, 12½ pounds to 50 gallons of water; *B*, with colloidal sulfur spray; *C*, with lime-sulfur, 1 gallon to 50 gallons of water; *D*, with a synthetic copper zeolite of 12-percent metallic content.

Another series of plots were used to compare various forms of sulfur, especially the spray materials, in order to ascertain their relative toxicity to the potato leafhopper on beans. Flotation sulfur paste at a strength of 8 pounds to 50 gallons of water (fig. 20, *A*) gave an excellent reduction of the population and a satisfactory control. The flotation sulfur dust (fig. 20, *B*), however, was not satisfactory and gave only a partial reduction. The particles of this material are very small and under certain conditions it is difficult to keep them on the plant for any length of time, which may account for its lack of toxicity as compared to the flotation paste. When dusting sulfur (fig. 20, *C*) was used the rate of reduction was slower but the control was satisfactory. The wettable sulfur in spray form at the strength of 5 pounds to 50 gallons of water (fig. 20, *D*) gave excellent reduction of the population and was a satisfactory control material. Dry-mix spray, 12½ pounds to 50 gallons of water (fig. 21, *A*), gave excellent population reduction and control. The dry mix was prepared with 8 pounds of flowers of sulfur, 4 pounds of hydrated lime, one-half pound

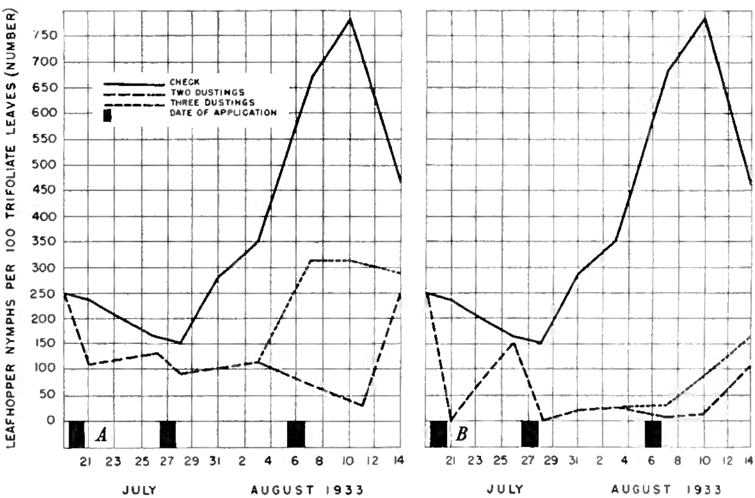


FIGURE 22.—Experiments on the control of *Empoasca fabae* on beans: *A*, With flotation sulfur and 0.5-percent pyrethrum dust, 95–5; *B*, with 0.05- and 0.5-percent pyrethrum dusts, 95–5.

of calcium caseinate, and 50 gallons of water. Colloidal sulfur (fig. 21, *B*) used on another plot gave only a partial reduction in numbers and unsatisfactory control. The curve in this case resembled more the curve obtained by the use of pyrethrum extract, and this colloidal form failed to show residual value. Lime-sulfur spray at the strength of 1 gallon to 50 gallons of water (fig. 21, *C*) gave a decided reduction in numbers but produced a curve similar to that of the pyrethrum extract. Plant injury frequently results from the use of this material, but as a rule the insects are satisfactorily controlled.

During these experiments it was also thought advisable to use a synthetic copper zeolite which had a metallic copper content of 12 percent. Used as a spray at a 1–to–200 strength (fig. 21, *D*) it proved unsatisfactory and gave only a partial reduction of the population.

In another series of experiments six plots were treated with different combinations of sulfur and pyrethrum dusts. Both flotation sulfur (figs. 22, *A*, and 23, *C*) and dusting sulfur (fig. 23, *A*, *B*) were used in combination with different grades of pyrethrum dust, and three grades of pyrethrum dust were used in combinations (figs. 22, *B*, and 23, *D*). The natural populations were variable in some of these plots, and it is therefore difficult to gain a comparison of the relative toxicity of these different combinations. If a general statement could be made, it would seem that the sulfur-pyrethrum combination gave

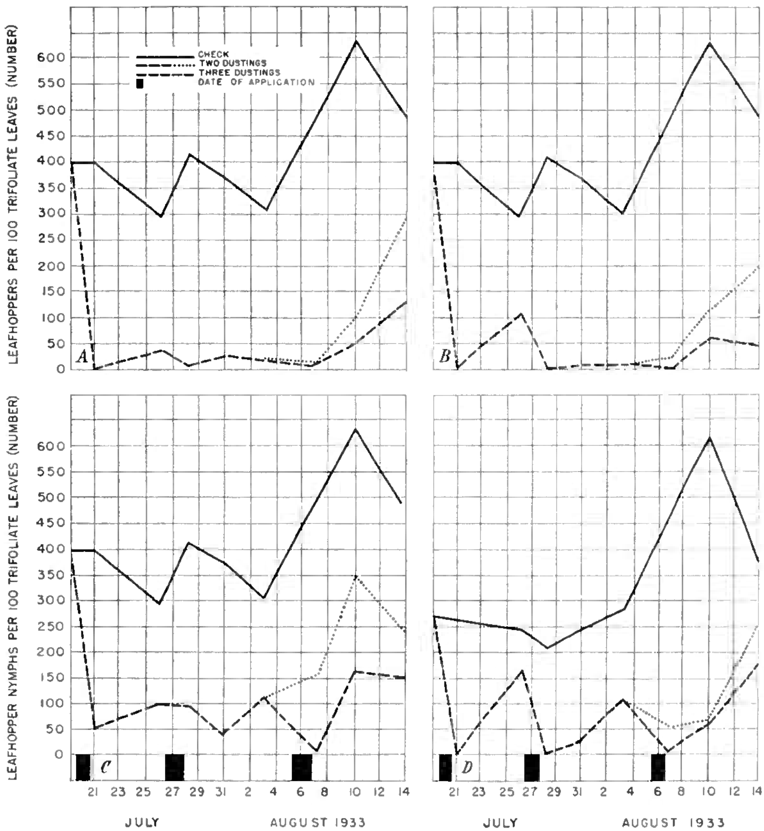


FIGURE 23.—Experiments on the control of *Empoasca fabae* on beans by use of the following dusts: *A*, Sulfur and a 1-percent pyrethrum dust, 95–5; *B*, sulfur and a 0.5-percent pyrethrum dust, 95–5; *C*, flotation sulfur and a 1-percent pyrethrum dust, 95–5; *D*, with 0.05-percent pyrethrum and 1-percent pyrethrum, 95–5.

excellent population reductions and that sulfur had a good residual control value, tho dusting sulfur apparently being superior to the flotation sulfur. The combinations of pyrethrum dusts alone, when charted, gave the saw-toothed type of curve, which is characteristic of the pyrethrum sprays. In these combinations exhausted flowers of pyrethrum containing 0.05 percent of total pyrethrins was used as the diluent.

Another series of experiments were performed in which pyrethrum dusts alone were used, dusting sulfur alone, and various combinations of dusting sulfur and pyrethrum. The two combinations of pyrethrum dusts (fig. 24, *A*, *B*) gave decided reductions in the populations but showed no residual toxic value. The declining populations in the check in each case cause the other curves to indicate what might be interpreted as residual value but which apparently was not. The dusting sulfur alone (fig. 25, *A*) gave a slow, rather gradual reduction

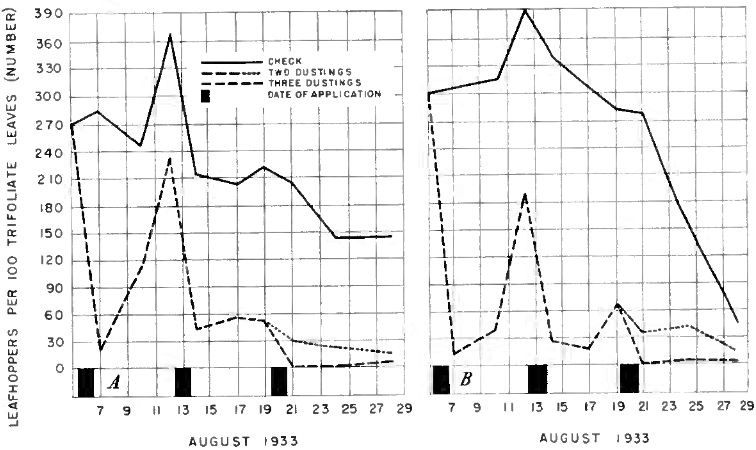


FIGURE 24.—Experiments on the control of *Empoasca fabae* on beans: *A*, By dusting with 0.05- and 1-percent pyrethrum, 95–5; *B*, by dusting with 0.05- and 0.5-percent pyrethrum, 95–5.

with the exception of a decided increase just before the second application.

Six different combinations of dusting sulfur and pyrethrum dusts were used, containing 1 (fig. 25, *C*), 0.5 (fig. 25, *B*, and *E*), and 0.05 percent (fig. 25, *D*, and *F*) of pyrethrins. Sulfur with a 1-percent pyrethrum dust at 95–5 gave a curve differing little from that when the proportions were 90–10. There seems to be little doubt that 5 parts of either a 1.0-percent or 0.5-percent pyrethrum with 95 parts of sulfur is sufficient to cause an immediate reduction of the leafhoppers present. The question of having the quantity of sulfur necessary to produce a sufficient residual value is, therefore, the more important problem. There is a possibility of added value due to the physical effects produced by the almost inert material in the pyrethrum dust containing 0.05 percent of pyrethrins. Combinations of sulfur and this grade of pyrethrum were therefore used and the results indicate that 25 percent of this grade of pyrethrum (fig. 25, *D*) contains a sufficient quantity of pyrethrins to give immediate reduction in the population, and the additional sulfur used, over the 50–50 combination (fig. 25, *F*), gives a much better residual value.

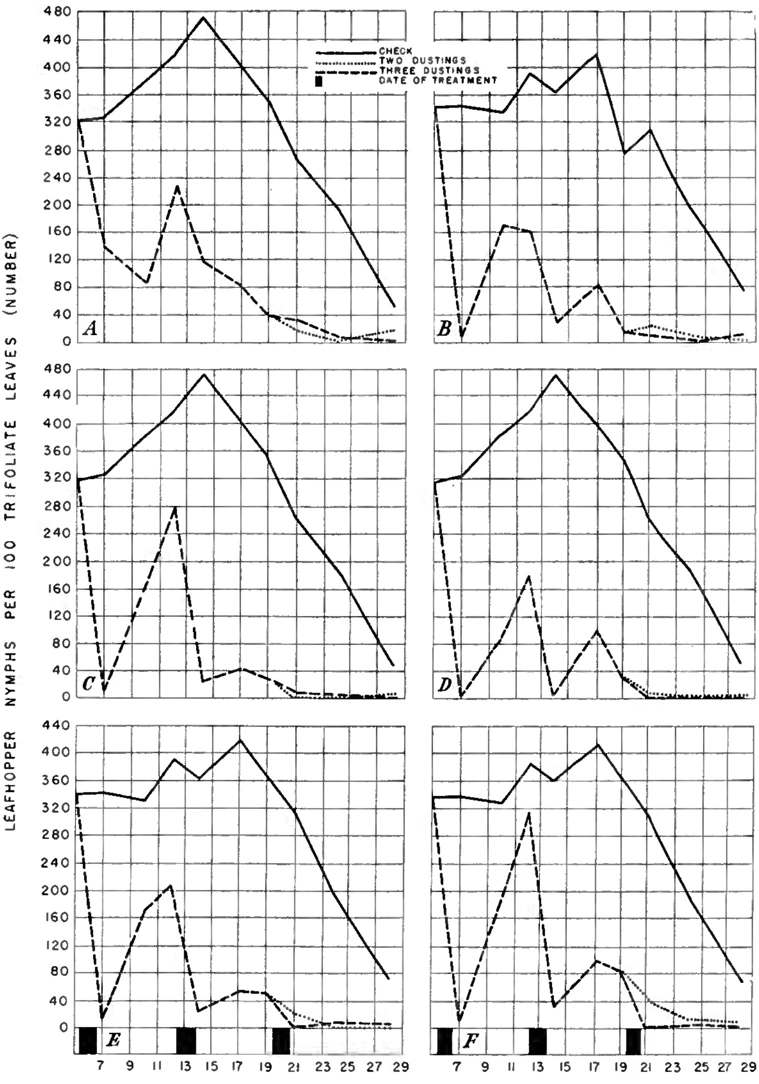


FIGURE 25.—Experiments on the control of *Empoasca fabae* on beans by use of the following dusts: *A*, Sulfur alone; *B*, sulfur and pyrethrum (0.5 percent), 90–10; *C*, sulfur and pyrethrum (1 percent), 90–10; *D*, sulfur and pyrethrum (0.05 percent), 75–25; *E*, sulfur and pyrethrum (0.5 percent), 95–5; and *F*, equal quantities of sulfur and a 0.05-percent pyrethrum dust.

SUMMARY

Field-plot tests in the control of *Empoasca fabae* as a bean pest were carried on with several materials during 1926–28 and 1932–33. Excellent immediate toxicity was obtained with various pyrethrum sprays but these lacked residual effect. Bordeaux mixture showed no immediate toxicity but had excellent residual effect and a delayed toxicity. Nicotine and oil sprays gave only partial control. Several other materials were unsatisfactory as control measures.

Leafhoppers placed on plants previously treated with bordeaux mixture died in 4 or 5 days. Leafhoppers covered with this material and placed on untreated plants were not killed. Leafhoppers confined to feeding on unsprayed surfaces of sprayed leaves died in from 5 to 10 days with symptoms of bordeaux poisoning. The lack of a contact factor and the slow effect on the leafhoppers gave a strong indication that the insects were obtaining copper compounds in some form from the plant liquids upon which they fed or were affected by a physiologic product of the plant induced by applications of bordeaux mixture.

Bordeaux poisoning was characterized in the leafhoppers by a replacement of the normal green by a yellowish color, and by weakening, inactivity, and motor paralysis accompanied by partial or faulty ecdysis. Small nymphs are more readily killed than large nymphs or adults.

Leafhoppers were fed through membranes upon various dilutions of copper sulfate with and without the addition of a 5-percent sugar solution, and upon various dilutions of calcium hydrate. The copper sulfate solutions, both with and without sugar, showed high toxicity to the leafhoppers and were from 10 to 20 times as toxic as lime solutions.

When the leafhoppers obtained copper compounds only by feeding on plants whose roots were placed in copper solutions, they were easily killed by the plant juices. These leaves were removed and copper was found in the expressed juices.

Lime sprayed upon plants had no toxic effect on the leafhoppers.

The supernatant liquid of bordeaux mixture was not toxic to leafhoppers and showed no soluble copper when tested by a method which should detect copper in the dilution of 1–50,000.

A relatively small percentage of copper sulfate in the bordeaux spray solution is effective for leafhopper control.

Bordeaux residue applied to filter papers in glass funnels and exposed to atmospheric conditions for several weeks failed to release soluble copper to the rain water which passed over it, but rain water collected from plants sprayed with this solution showed positive tests for soluble copper.

Sugar solutions and expressed plant juices readily dissolved soluble copper compounds from dry bordeaux residue through capping (permeable) membranes. Distilled and tap water gave only negative results.

Refractometer readings of plant sap from sprayed and unsprayed bean and potato plants showed that as a rule the sugar content of the treated plants is lowered for 1 or 2 days after treatment below that of the untreated plants, but that it soon rises in the treated plant above that of the untreated, and usually remains higher for 2 or 3 weeks.

The plant physiology is changed by the presence of bordeaux mixture in such a way that a temporary resistance is produced in the plant which produces toxicity to the leafhoppers feeding upon it for a period of several days after treatment.

Tests of various preparations of sulfur were made by four methods, following somewhat the plan of the tests with bordeaux mixture. Leafhoppers were sprayed with the sulfur, plants and insects were sprayed together, plants were sprayed with sulfur and the leafhoppers placed on them from 1 to 26 days afterward, and the sulfur preparations were exposed in shallow dishes directly under the infested plant, the whole being covered by a bell jar. Only slight toxicity was caused by the direct spray on the insect, and in the case of the materials exposed under the plants the mortality was not greater than in the check. When the insects fed on sprayed plants from the time of spraying up to 26 days afterward the mortality was high as compared with the check.

Pyrethrum extract kills practically all the leafhoppers present at any time it is applied, but the leafhoppers hatching from the eggs in the leaf tissue cause a rebuilding of the population.

When tobacco dust was mixed with sulfur, the reduction in populations was no greater than when sulfur was used alone.

Copper-lime dust was of little value under Ohio conditions and could not compare with bordeaux mixture or some of the sulfur-pyrethrum combinations.

Bordeaux mixture used extensively on field plots gave excellent reductions of the populations and showed a residual value somewhat greater than that of any other material used, but when 3–4½–50 bordeaux mixture was combined with a 2-percent white oil the residual value was greatly reduced.

Among the sulfur materials, flotation sulfur paste, 8 pounds to 50 gallons of water, wettable dry sulfur spray, 5 pounds to 50 gallons of water, and dry-mix spray, 12½ pounds to 50 gallons of water, gave excellent reduction of populations. Dusting sulfur gave more gradual but good control. Liquid lime-sulfur gave fair control, but some plant injury, and showed the lack of residual effectiveness during a part of the time.

Sulfur dust used with pyrethrum containing either 0.5 or 1.0 percent pyrethrins in combinations of 90–10 or 95–5 usually showed very good killing qualities and a good residual value.

When sulfur dust was used with pyrethrum containing 0.05 percent pyrethrins in combinations 50–50 and 75–25, both combinations had good toxic qualities, but the 75 percent of sulfur was superior regarding residual value to the 50 percent of sulfur.

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